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БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY

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THE ROLE OF CARBOHYDRATE-PHOSPHORUS METABOLISM IN THE PRODUCTION OF THE EXCITATION CURRENT IN SKELETAL MUSCLE

A. S. Mozzhukhin (Leningrad)

(Received December 13, 1956. Presented by Academician L. A. Orbeli)

In recent years several research workers have studied the role of metabolism in the production of bioelectrical phenomena. A relationship has been demonstrated between metabolism on the one hand and both "stationary" electrical processes of long duration (currents of injury, salt-induced or temperature currents) and "dynamic" rapidly developing electrical processes (excitation currents of nerve and muscle tissue).

A relationship has been shown between the current of injury of skeletal muscle and nerve [1, 5, 6, 9, 11, 13], salt-induced currents [2] and temperature currents [4] and the carbohydrate-phosphorus metabolism.

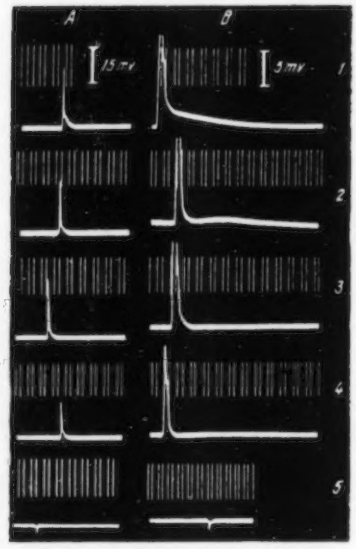

Fig. 1. The effect of poisoning the muscle with monoiodiacetate on the excitation EMF.
Time marker) 0.05 seconds. 1) initial recording; 2) after resting for 30 minutes in monoidoacetate solution; 3) after 60 minutes; 4) after 90 minutes; 5) after resting for 120 minutes in the same solution.

It has been found that the magnitude and form of the excitation current of skeletal muscle depend on carbohydrate metabolism [3, 8, 12] and also that disturbance of glycolytic processes affects the electroretinogram and the electrocardiogram [7]. During poisoning of the skeletal muscle of the frog with monobromoacetic acid, changes are found in the after-potentials only, with no change in the action current peak [12].

Changes in the blood sugar concentration in cats were found to be accompanied by changes in the excitation current peak in skeletal muscle [3]. In earlier work on the study of the effect of poisoning the skeletal muscle of the frog with monoiodoacetic acid on the excitation current, no changes in the latter were found [10]. Poisoning the skeletal muscle of the frog with sodium fluoride leads to a reduction in the amplitude of the excitation current [8].

The object of the present investigation was to study further the role of carbohydrate metabolism in the production of the excitation current of skeletal muscle.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated sartorius muscles of autumn frogs. The sartorius muscle was carefully dissected, and placed on a paraffin wax block in a humid chamber for detection of the current. To avoid distortion of the recording during contraction, the muscle was fixed in a slightly stretched position. The excitation current was taken from damaged and undamaged areas of the muscle by

means of nonpolarizing ($Zn-ZnSO_4$) electrodes to a loop electrocardiograph. With leads of this type the excitation current was always monophasic.

Direct stimulation of the muscle was carried out by means of a neon interrupter. The duration of each impulse was 1 millisecond and the intervals between the impulses were of one second. The current strength was always over threshold (the muscle gave a maximum contraction).

The distance between the stimulating platinum electrodes was 1-1.5 mm, the distance between the stimulating and take-off electrodes was 10 mm, and that between the take-off electrodes was 5-10 mm. The short duration of the stimulating current and the position of the electrodes in which the excitation current and the stimulation current (both monophasic) could be arranged to have their apices on opposite sides, enabled the currents to be recorded without any distortion due to the "loop" of the stimulating current.

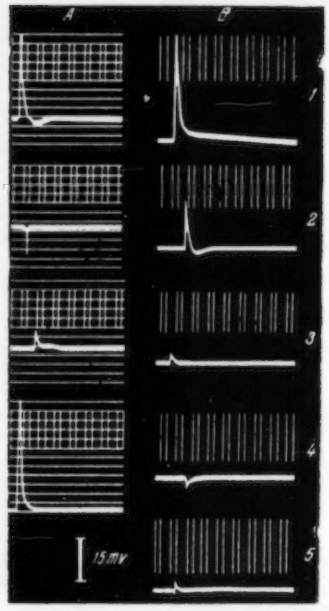

Fig. 2. The effect of poisoning the muscle with cyanide on the excitation EMF.
Time marker) 0.05 seconds. A) Poisoning with a 0.1% solution: 1) initial recording; 2) after resting for 30 minutes in cyanide solution; 3) after resting for 30 minutes in Ringer solution; 4) after 60 minutes. B) Poisoning with a 0.01% solution: 1) initial recording; 2) after resting for 30 minutes in cyanide solution; 3) after 60 minutes; 4) after 90 minutes; 5) 30 minutes after resting in Ringer solution.

In the intervals between recording the bioelectric currents the muscles were kept in Ringer solution, to which various substances could be added as necessary.

Changes in the excitation current were studied:

1) during poisoning of the muscle with a solution of sodium monoiodoacetate (0.002%), a solution of sodium fluoride (0.042%) and a solution of sodium cyanide (0.1%);

2) during recovery of the current after rinsing the poisoned muscles with Ringer solution, a 0.002% solution of vitamin B_1 (in monoiodoacetate poisoning of the muscle) and a 0.11% solution of sodium pyruvate (in sodium fluoride poisoning of the muscle);

3) during rhythmic stimulation of the muscle for 10-20 minutes and subsequent keeping of the muscle for 30 minutes in Ringer solution or in vitamin B_1 solution (for muscles poisoned with monoiodoacetate).

EXPERIMENTAL RESULTS

The figures which are given show the changes in the excitation current of the sartorius muscle during monoiodoacetate poisoning (Fig. 1) and cyanide poisoning (Fig. 2). It can be seen that during poisoning of the muscle changes took place in both the magnitude of the peak of the action current and in the after-potential. The size of the peak and also of the after-potential gradually fell and then completely disappeared. Under these circumstances the after-potential disappeared even when the action-potential peak was hardly altered (the first 15-30 minutes from the beginning of the experiment). The opposite muscle, kept in Ringer solution for 2-4 hours, showed no changes in the excitation current.

The presence of vitamin B_1 in the monoiodoacetate solution caused a decrease in the changes in the peak of the excitation current. The presence of pyruvate in the fluoride solution had no such effect.

Rhythmic stimulation by the current for 10 minutes of muscles kept for 1 hour in solutions of sodium monoiodoacetate and fluoride led to a considerable decrease in the peak of the excitation current, and stimulation for 20 minutes – to the complete disappearance of the peak. The opposite muscles, kept in Ringer solution showed no such changes.

TABLE

The Effect of Sodium Pyruvate in a Solution of Sodium Fluoride on the Rate of Fall of the EMF of Injury in the Skeletal Muscle of the Frog (mean of 10 experiments)

Time (in hours)	Control		0.042% sodium fluoride		0.042% sodium fluoride and 0.11% sodium pyruvate	
	absolute magnitude EMF, mv	as a % of the initial value	absolute magnitude EMF, mv	as a % of the initial value	absolute magnitude EMF, mv	as a % of the initial value
0	52	100	55	100	60	100
1	37	71	36	65	44	73
2	34	65	29	53	40	66
3	30	57	24	43	37	61
4	29	55	21	38	35	58
5	28	54	17	30	34	56
6	27	52	15	27	33	55

Rinsing the muscles poisoned with monoiodoacetate with vitamin B_1 solution led to some degree of acceleration of the partial re-establishment of the peak of the excitation current, compared with the muscle on the opposite side, rinsed with Ringer solution. No difference could be found between the effect of rinsing with Ringer solution or with sodium pyruvate solution, in the case of a muscle poisoned with sodium fluoride.

It follows from the experiments described that disorders of both the anaerobic phase of carbohydrate metabolism (poisoning with monoiodoacetate and fluoride) and of the processes of oxidation (cyanide poisoning) led to a decrease or even to complete disappearance of the excitation currents of skeletal muscle. These disorders were to a large extent reversible: rinsing the poisoned muscles with Ringer solution led to some degree of re-establishment of the excitation currents. The excitation currents of muscles poisoned with monoiodoacetate and fluoride could be greatly reduced (or even made to disappear completely) by rhythmic stimulation of the muscles for 10-20 minutes, and they were restored once again if the muscles were kept for 30 minutes in Ringer solution or in vitamin B_1 solution (for a muscle poisoned by monoiodoacetate).

All the changes mentioned in the excitation currents were not directly associated with the development of a contracture in the poisoned muscles, since: 1) the excitation currents disappeared roughly 30 minutes after the contracture had developed; 2) after poisoning of the muscle with sodium cyanide and the development of the corresponding changes in the excitation currents, generally no contracture developed (at least during the time interval of our investigation); 3) placing a muscle poisoned with monoiodoacetate in vitamin B_1 solution led to some increase in the excitation currents, although vitamin B_1 did not prevent the development of a contracture [6].

All this suggests that in this particular case we were in fact dealing with changes in the excitation current depending mainly on changes in carbohydrate metabolism and not on disturbances of the structure of the muscle.

It is also important to mention that disturbance of carbohydrate metabolism does not affect absolutely equally the excitation current and the current of injury. Poisoning of skeletal muscle with sodium cyanide does not lead to any perceptible change in the current of injury [5, 6], whereas it does have a considerable effect on the magnitude of the excitation current. The presence of pyruvate in the sodium fluoride solution completely paralyzed the effect of the latter on the current (EMF) of injury (see Table) but did not affect the changes in the excitation current (EMF).

The findings described suggest that the various phases of carbohydrate metabolism have different importance for the existence of the excitation current and the current of injury.

SUMMARY

A disturbed carbohydrate-phosphorus metabolism produced by the poisoning of an isolated sartorius muscle in a frog with monoiodoacetate fluoride and cyanide results in a variation and complete disappearance of the

skeletal muscle excitation currents. The variation is manifest in the gradual decrease (and at times also in the change of the form) both of the trace potentials and the peak value of the excitation current. The changes begin with the trace potentials. The data obtained allow us to presume the necessity of certain differences in the value of individual phases of the carbohydrate-phosphorus metabolism for the presence of excitation and injury currents.

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FREQUENCY OF THE RHYTHMIC CONTRACTIONS OF THE SMALL INTESTINE

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(Received September 2, 1958. Presented by Active Member Acad. Med. Sci. USSR, V. N. Chernigovskii)

When studying the mechanisms of nervous control of the motor function of the intestine and the paths of transmission of excitation to its neuromuscular apparatus we used the method of division of the intestinal tube, and during chronic experiments we found that division of the wall of the intestine leads to a severe fall in the frequency of the rhythmic contractions distal to the point of division [1].

In view of the important role of rhythmic contractions in the process of digestion and absorption, and also of the importance attached to it by the gradient theory of intestinal polarity [2, 3], we made a detailed study of the frequency of the rhythmic contractions.

EXPERIMENTAL METHOD

The investigation was carried out in the form of chronic experiments on 26 dogs (fasting and fed) with fistulae of the stomach, duodenum and jejunum. Two of the animals also had a fistula of the ileum 30 cm proximal to the ileocecal sphincter. Experiments were performed before and after division of the intestinal tube at predetermined levels in normal dogs and in dogs after ligation of the vagus and splanchnic nerves.

Patency of the intestine was ensured by means of external anastomoses or by the formation of entero-enterostomies. Movements of the intestine were recorded by a graphic method using balloons.

EXPERIMENTAL RESULTS

In the healthy dogs the frequency of contraction of the duodenum and the proximal jejunum varied between 17-19, and in some of the experimental dogs – between 18-20 contractions per minute. The frequency of rhythmic contraction of the jejunum at a distance of 40-70 cm caudal to the duodenal ligament (ligament of Treitz) was the same as in the initial part of the jejunum, or slightly lower (by 0.5-1 contraction per minute). In an area of the intestine situated 20-30 cm proximal to the ileocecal sphincter it was 12-13.5, and in some dogs 12.5-14 contractions per minute.

After division of the intestinal wall at a distance of a few centimeters (2-5 or 8-10 cm) caudal to the duodenal ligament (passage of food was ensured outside the experiment through an external anastomosis joining the oral and caudal sections of the bowel), a sharp decrease was observed in the frequency of rhythmic contractions of the jejunum caudal to the division, whereas in the neighboring area, oral to the division, the normal frequency of rhythmic contraction was maintained.

Whereas in the normal dogs the upper part of the jejunum contracted at the rate of 18-20 per minute, after division of the intestine the frequency of these contractions in areas situated caudal to the divison was 12.8-13.2 per minute in the first few days after the operation. On the 10th day after operation it rose and after

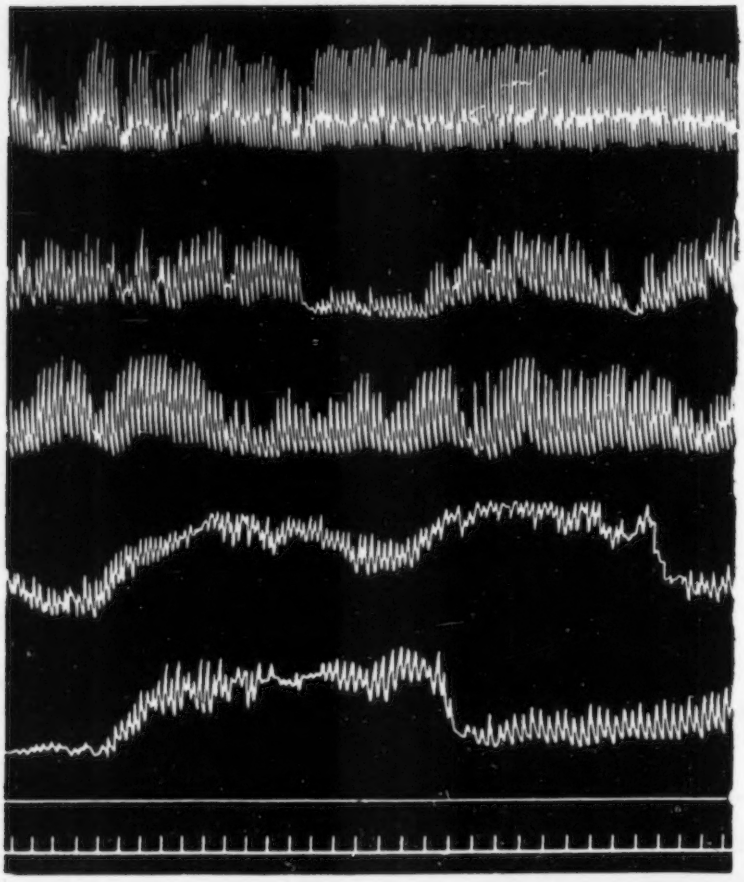

Fig. 1. Frequency of contraction of different areas of the small intestine, recorded simultaneously, in a chronic experiment on the dog Lotsman after division of the intestine at various levels.
Significance of the curves (from above down): contractions of the lower third of the duodenum; contractions of the jejunum distal to the division at the ligament of Treitz (Thiry-Vella segment); contractions of the jejunum distal to a division carried out 30-35 cm caudal to the ligament of Treitz; contractions of the terminal part of the ileum proximal to a division made 25 cm from the ileocecal sphincter; contractions of the terminal part of the ileum distal to this division; line of the stimulation marker; time marker (17 seconds).

20-40 days it reached 13.5-14.7 contractions per minute. No further tendency for the frequency of contraction caudal to the division to increase was observed.

Recording of the movements of the jejunum at distances of 25, 45 and 65 cm caudal to the division showed that a sharp fall in the frequency of rhythmic contractions was observed not only in the caudal areas adjoining the site of division, but also more distally along the bowel, at least for 60-70 cm.

For this reason the statement of Alvarez [3] that the fall in frequency of contraction of the jejunum after novocainization or compression of the bowel at the level of the duodenal ligament, as observed in Douglas's

experiments [6], was characteristic of only the segment of jejunum adjoining the site of compression or novocainization, does not correspond to the facts. The unsoundness of this view could not have been revealed by Douglas's experiments with division and re-anastomosis of the intestine above the exteriorized loop (by Biebl's method [4, 5]), for in recording the contractions of the intestine, Douglas was able to utilize only a small area of the small intestine in his experiments.

Thus in the first few days after division of the bowel at the duodenal ligament the frequency of rhythmic contraction of the upper part of the jejunum, caudal to the division, was no higher than the frequency of contraction of the terminal part of the ileum, i.e. there was no sign of a gradient of frequency of contraction caudal to the division. However, 20-40 days later, the gradient was re-established to a definite degree. Whereas, however, in the normal animal the difference between the frequency of contraction of the upper areas of the jejunum and the ileum at a distance of 20-30 cm oral to the ileocecal sphincter was 5-6 contractions per minute, from 1-1½ months after division of the wall of the intestine at the level of the duodenal ligament the difference was only 0.8-1.5 contractions per minute. Such a fall in the frequency of the rhythmic contractions caudal to the division of the bowel wall was observed in all the experimental dogs.

1 month 11 days after division of the bowel near the duodenal ligament, in one dog (Lotsman) a second division of the bowel wall was made at a distance of 30-35 cm caudal to the first. The area of bowel between the first and second divisions was exteriorized in the manner of a Thiry-Vella segment, and the passage of food along the intestine was assured, outside the experiment, by an external anastomosis joining the duodenum to the initial part of the segment of jejunum caudal to the second division. The area of intestine caudal to the second division (1 month after this operation) had a frequency of 13.2-14.2 contractions per minute, whereas in the normal animal before division of the bowel this area had a frequency of 17-19 contractions per minute. The frequency of contraction of the duodenum at this time in the dog was 18-20 contractions per minute; the frequency of contraction at a distance of 20-30 cm oral to the ileocecal sphincter was 12-13.5 contractions, and in the Thiry-Vella segment — 13.5-14.7 contractions per minute (Fig. 1).

Hence after the second division also the dominant area in respect to frequency of contraction was the oral part of the bowel, situated caudal to the site of division, but here, too, there was not such a severe fall in the gradient of frequency in the direction of the terminal part of the ileum as was observed in the normal animal.

A third division of the bowel wall in the dog Lotsman was carried out at a distance of 25 cm oral to the ileocecal sphincter. The oral segment of the ileum was connected, outside the experiment, to its caudal segment by means of a second external anastomosis. 10 days after the third division of the bowel, and on subsequent days, the frequency of rhythmic contraction of the proximal segment of the ileum was 12.5-14 contractions per minute, whereas in the distal segment 9.5-11.6 contractions per minute were observed (Fig. 2). The frequency of contraction of the intestine distal to the division of the bowel was equally low in all the fed or fasting animals, both at the beginning and the height of the process of digestion.

In dogs after bilateral transdiaphragmatic division of the vagus nerves, and in dogs after division of the splanchnic nerves and extirpation of the sympathetic chains in the lumbar region, the frequency of rhythmic contractions of the small intestine was no different from that observed under normal conditions (before division of the nerves).

In order to exclude completely any nerve supply to the intestine from the vagus nerves, after the normal frequency of contractions had been established in three dogs with fistulae of the stomach and jejunum, a bilateral vagotomy was performed in the neck. The frequency of rhythmic contractions in the small intestine after the operation was the same in all three dogs as before. Occasionally, in the first 7-10 days after the operation a pyloric rhythm was observed for 10-20 minutes, after which the normal frequency of contraction of the jejunum was restored.

If in the dogs in which the splanchnic or vagus nerves or both were divided, the intestine also was divided at the level of the duodenal ligament or slightly caudal to it (5-8 cm), the frequency of rhythmic contraction of the jejunum caudal to the division fell sharply (12-13 contractions per minute), and 1-2 months after this operation it reached a value of not more than 14-14.5 contractions per minute.

The results obtained showed that the frequency of rhythmic contraction of different segments of the small intestine in the normal animal is determined not by the extrinsic innervation and not by the food passing through

the lumen of the bowel, but by the activity of the area or of the structures present in the walls of the digestive tube, oral to the ligament of Treitz.

It was necessary to find out which particular area of the digestive tube oral to the ligament of Treitz was responsible for the rhythm of the contractions of the small intestine. In order to solve this problem we employed the method of division of the bowel and subsequent formation of an end-to-end anastomosis, since after such an operation in our experiments we always observed the same fall in the frequency of the contractions distal to the anastomosis as after division of the intestine and subsequent restoration of the patency of the bowel by means of an external anastomosis [1].

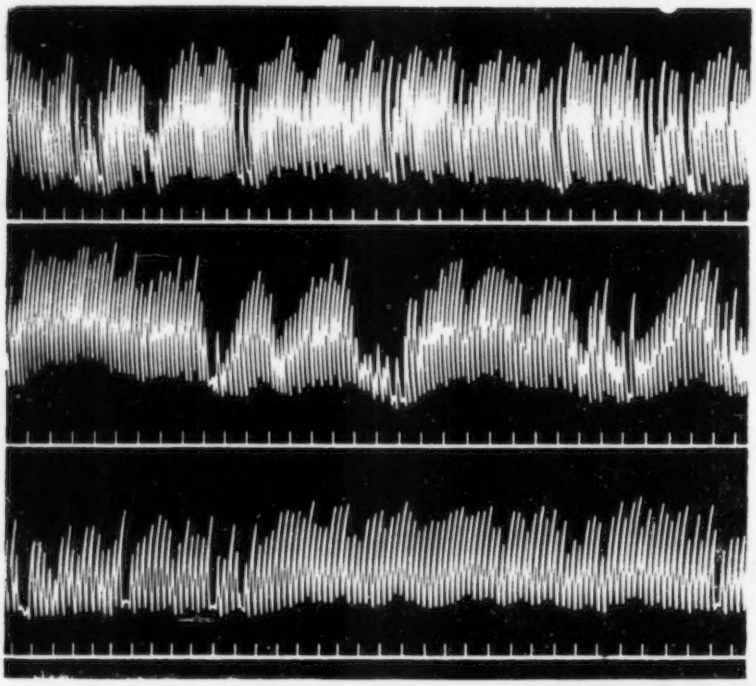

Fig. 2. Contractions of the jejunum in an area situated 15-18 cm caudal to the ligament of Treitz.
Significance of the curves (from above down): contractions in a normal dog; contractions one month after division of the bowel oral to the attachment of the body of the pancreas to the intestine and the formation of an end-to-end enteroenterostomy; contractions 4 months after division of the duodenum distal to the attachment of the pancreas and the formation of an end-to-end entero-enterostomy; time marker (15 seconds).

After division of the digestive tube at the junction of the pylorus and duodenum, or division of the duodenum above the place of attachment of the pancreas, followed by the formation on an end-to-end enteroenterostomy (experiments on 6 dogs) the frequency of contraction of the upper part of the jejunum was 17-19 contractions per minute, i.e. it was normal. Division of the bowel at the lower border of the attachment of the pancreas, followed by the formation of an enteroenterostomy at this point (experiments on 3 dogs) led to a fall in the frequency of rhythmic contractions of the jejunum. Whereas before division and anastomosis the frequency was 18-20 contractions per minute, from 1-1½ months after this operation it was 13.5-15 contractions per minute (see Fig. 2), and for the next 6 months no increase in the frequency of the contractions was observed.

It can be seen from the results of our experiments that the frequency of the rhythmic contractions of the intestine distal to the division fell sharply only when the division was made below the attachment of the body of the pancreas to the duodenum, and it remained normal in all the experiments after division of the bowel proximal to the attachment of the pancreas to the duodenum.

Thus the area of the duodenum to which the pancreas is directly applied, or some structure present in that area, evidently plays a leading part in determining the normal frequency of rhythmic contraction of the duodenum and jejunum, setting the rhythm (the "pacemaker" of the rhythm) of the intestinal contractions.

The frequency of the rhythmic contractions of each particular segment of the intestine is not a property of the neuromuscular or muscular apparatus of that segment, as asserted by Alvarez [3]. It may be determined by structures which lie outside that segment; since the intrinsic rhythm of the segments of the jejunum is considerably below that observed in the normal animal. The "pacemaker" of the rhythm, which we discovered, is of practical as well as theoretical interest, in connection with the often performed operation of resection of small areas of the small intestine, since enteroanastomosis does not restore the pathways by which the segments of the intestine are normally joined to each other.

This can be seen from the fact that end-to-end or side-to-side enteroenterostomy does not restore the normal frequency of contraction of the intestine caudal to the division. The rhythmic contractions of the jejunum caudal to a resection are maintained at a low frequency (13.5-14.6 contractions per minute) 5 years after operation.

It must be pointed out that in all the experimental dogs the Thiry-Vella segments of the jejunum had a frequency of rhythmic contraction of 13.5-14.7 per minute, whereas in normal dogs these areas of the small intestine had in some cases a frequency of 17-19 and in others a frequency of 18-20 contractions per minute. This fact has remained unnoted.

The fall in the frequency of rhythmic contraction of the terminal part of the ileum caudal to the division, compared with the frequency in this area in the normal animal, showed that the rhythm of the contractions of the ileum evidently depended on structures which were situated proximal to the point of division of the ileum which we selected, for division of the bowel at the level of the ligament of Treitz had no effect on the frequency of the contractions of the ileum.

The results obtained are of great importance for an understanding of the mechanisms of peristalsis. The gradient theory of peristalsis (polarity) of the intestine is unacceptable even in the form in which it is described in the latest edition of Alvarez' monograph [3], for it does not explain the physiological connection between the gradients and the caudal advancement of the peristaltic wave [1, 8]. In addition, according to this theory, the wave of excitation arising at any point in the intestine must spread to the side of its greatest excitation, but in fact the peristaltic wave spreads along the intestine in a caudal direction i.e. toward the side of its least excitation.

Thus the wave of excitation spreads not with but against the gradient of excitation.

Our experimental results show that the area of the duodenum to which is attached the pancreas, or structures present in this area, fulfil the function of "pacemaker" for the rhythm of contraction of the duodenum and jejunum.

According to preliminary findings, the "pacemaker" for the rhythm is located in the area of the outlet of the bile duct and the accessory pancreatic duct into the intestine. From this "pacemaker" for the rhythm, impulses constantly pass out to the neighboring areas of the intestine and spread further distally along the intestine.

In consequence of the presence of a "pacemaker" or of "pacemakers" for the rhythm, it is possible to show the cause of the spread of the peristaltic waves in a caudal direction. Impulses constantly passing out from the "pacemaker" do not allow the spread of the wave of excitation in the oral direction, because in case of spread of such a wave (an antiperistaltic wave) it is very rapidly or even immediately extinguished by the phase of diminished excitation after each descending impulse. A similar mechanism has also been suggested by Thomas [8].

The rate of extinction of the antiperistaltic wave depends on the strength of the wave of excitation and the

depth of refractoriness which follows behind the excitation produced by the descending impulse from the "pacemaker" of the rhythm. The descending impulses from the "pacemaker" of the rhythm enable the wave of excitation to spread in a caudal direction and bring about the strengthening and acceleration of the rapid peristaltic waves. Extinction of a peristaltic wave, which may take place at any level of the intestine, can be explained by the diminished excitation of the distal segments of the bowel or by active inhibition through the extrinsic nerves of the intestine in response to the action of stimuli on particular segments of the bowel or on divisions of the gastrointestinal tract.

The position of the "pacemaker" of the rhythm in the area of the bowel to which the pancreas is attached creates conditions in the initial part of the duodenum for the spread of the wave in a caudal direction (peristaltic waves) and in an oral direction (antiperistalsis). When the intestine is divided caudally to the "pacemaker" of the rhythm, the function of the latter is evidently taken over by the oral area of the intestine distal to the division, since the frequency of the rhythmic contractions of this area is greater than the frequency of contraction of the terminal part of the ileum [1].

SUMMARY

Chronic experiments were performed on dogs with divided intestine at the level of Treitz ligament. A sharp decrease in the frequency of jejunal rhythmical contractions (by 25%) caudal to the site of division was noted. Following intestinal division between the pylorus and duodenum, and 3-4 weeks after the division of the duodenum (oral to the attachment of the pancreas) and placing end-to-end enteroenterostomies – normal frequency of rhythmical contractions was noted, distal to the site of division. Division of the duodenum 0.5 to 1 cm caudal to the attachment of the pancreas resulted in a sharp and stable decrease in the frequency of intestinal rhythmical contractions distal to the cut. The presence of an intestinal contraction automatic "pacemaker" is inferred. found in the intestinal portion directly adherent to the pancreas. The functioning of such a pacemaker explains the mechanism governing the extension of peristaltic contractions in the caudal direction.

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THE RELATIONSHIP OF THE HYALURONIDASE AND ANTIHYALURONIDASE ACTIVITY OF THE RETINA TO THE INTENSITY OF A LIGHT STIMULUS

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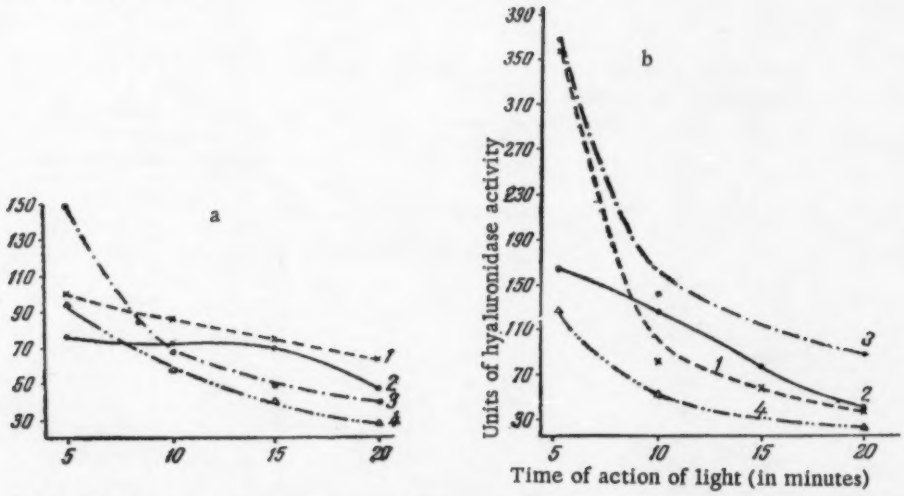
The enzyme hyaluronidase takes an active part in many processes in the body associated with the application of the permeability of the "basic substance" of connective tissue and with a reduction in the viscosity of certain fluids. The hyaluronidase of bacteria is widespread as a factor facilitating their entry into the animal body. A connection has been found between hyaluronidase and fertilization (McClean [6]; Duran-Reynals [5]), and it has been found to affect the permeability of the capillaries (B. N. Mogil'nitskii [3]).

The hyaluronidase of the ciliary body is important in the regulation of the circulation of the intraocular fluid and also in the maintenance of the transparency of the optic media of the eye (Meyer and Palmer [7]; Meyer, Smyth and Gallardo [8].

Kh. S. Koshtoyants [2] demonstrated that hyaluronidase took part in nerve excitation. We ourselves reported [1] the secretion of hyaluronidase by the retina of the frog.

In the present investigation we studied the connection between the hyaluronidase activity of the retina and its functional state, and also the causes of the fall in the hyaluronidase activity of the retina during prolonged, continuous action of a light stimulus.



Changes in the activity of the hyaluronidase secreted by the retina of frogs (a) and rabbits (b) depending on the intensity of the light stimulus and the time of its action. Conventional signs: 1) during strong stimulation by a 15 w lamp; 2) 1 w; 3) 40 w; 4) 150 w.

EXPERIMENTAL METHOD

The material used in the investigation was prepared from the enucleated eyes of animals (frogs and rabbits). By an incision along the limbus the posterior half was removed, consisting of part of the eyeball, in the form of a shallow cup inside which were the retina and the pigmented membrane. The final preparation from the eye was placed in a vessel containing a nutrient solution.

The activity of the hyaluronidase thus secreted was determined at definite intervals of time in the nutrient solution by means of viscosimetry, and was expressed in conventional units.

Determination of the activity of the antihyaluronidase factor was based on its power to reduce the activity of a standard solution consisting of testicular extract with a certain concentration of the enzyme hyaluronidase.

Nutrient salt solution taken from that in which the retinas were kept, and tested for antihyaluronidase activity, was mixed with testicular extract and allowed to stand for 15-20 minutes.

The hyaluronidase in the mixture was determined before and after this operation; the antihyaluronidase activity was estimated by the difference and was expressed as conventional units and as true units of concentration of enzyme and antifactor. We made use of the graph of the relationship between units of activity and the logarithm of the enzyme concentration, as drawn up by Swyer and Emmens [9].

TABLE 1

The Effect of Alternate Stimulation and Rest on the Hyaluronidase Activity of the Retina

Record no.	Hyaluronidase activity in conventional units							
	right retina				left retina			
	light	dark	light	dark	light	dark	light	dark
	exposure for 5 minutes							
97	149	30	127	33	24	130	30	116
98	120	50	106	47	39	128	47	120
99	134	46	110	46	32	110	43	105
100	137	20	106	18	35	106	18	104
101	118	23	95	22	30	98	17	92

TABLE 2

Hyaluronidase and Antihyaluronidase Activity of the Nutrient Solution with Illumination for 20 Minutes

Record no.	Without changing the nutrient solution		Nutrient solution with exposure for 10 minutes	
	concentration		concentration	
	of hyaluronidase	of antifactor	of hyaluronidase	of antifactor
44	—	—	1,09	2,12
56	1,57	1,80	1,35	1,18
59	1,66	1,32	1,37	1,66
108	1,43	0 76	1,22	1,64
110	1,68	2.10	1,60	2,00
113	1,35	1 40	1,35	1,60
118	1,41	1,70	1,26	1,60
119	1.40	1,80	1,30	2.00
Mean	1,50	1,55	1,32	1,80

TABLE 3

Hyaluronidase and Antihyaluronidase Activity of the Nutrient Solution in Succesive Determinations in the Course of 20 Minutes

Record no.	Enzyme	Exch. of nutrient solution		No change of nutrient solution
		5 minutes (the first)	10 minutes (the last)	
56	Hyaluronidase	2,50	1,35	1,57
	Antihyaluronidase	3,0	1,8	1,8
111	Hyaluronidase	2,13	1,35	1,35
	Antihyaluronidase	2,14	1,60	1,4

EXPERIMENTAL RESULTS

The retina of the frogs was stimulated by interrupted light, alternating every 5 minutes with darkness. In this way the repeated, brief action of the stimulus together with equal intervals of rest was achieved. The results of some of the experiments are shown in Table 1.

The hyaluronidase activity of the retina was increased during illumination and decreased when the retina was darkened. These results suggested that the hyaluronidase activity depends on the functional state of the retina.

The relationship of the hyaluronidase activity of the retina to the intensity of the light stimulus was next studied. The source of light used was electric lamps of different power: 1, 15, 40 and 150 w. The intensity of illumination created by the lamps was measured with an illuminometer, and was respectively 14-18, 140-240, 380-540 and 1143-1900 lux.

In 54 experiments with preparations of frogs' eyes and 21 experiments with preparations of rabbits' eyes it was shown that the activity of the secreted hyaluronidase depends on the intensity of the light stimulus.

With an increase in the intensity of illumination from 14 to 540 lux the hyaluronidase acitivy increased. The highest hyaluronidase activity was observed at an intensity of illumination of 380-540 lux.

The results obtained may be compared with those of Z. V. Smelyanskii and V. V. Meshkova [4], according to whom an intensity of illumination of 400-500 lux is optimal for the eye.

A further increase in the intensity of illumination to 1143-1900 lux led to a considerable fall in the activity of the secreted hyaluronidase. We looked upon this as the manifestation of a specific physiological state of the nerve cells in response to the action of a strong stimulus.

In a series of experiments the activity of the secreted hyaluronidase was determined repeatedly, every 5 minutes during the interrupted action of light for 20 minutes (see Figure).

In 34 experiments with preparations from frogs' eyes and in 19 experiments with preparations from rabbits' eyes it was found that the prolonged, interrupted action of light gradually reduced the hyaluronidase activity in the nutrient solution. The rate of fall of this activity depended on the intensity of light used.

Exposure to a light stimulus of 14 to 240 lux gave, in the case of the frog's retina, a low initial hyaluronidase activity, which fell slowly and slightly during the 20 minutes of illumination. When the eye preparation was illuminated by 380-540 lux the rate of fall of hyaluronidase activity was noticeably altered: the high initial activity fell rapidly and considerably during the period of time.

A light stimulus of 1143-1900 lux also shoed a rapid fall in the initial activity.

In the experiments with the preparations from rabbits' eyes similar results were obtained (see Figure).

Thus the higher the initial activity of the hyaluronidase, the quicker its subsequent fall. We thought that the hyaluronidase activity of the solution containing the eye preparations illuminated interruptedly for 20 minutes would be higher than that of the solution in which the preparation had been illuminated only for the first 5 minutes. The activity was found to be lower, however, and in the majority of the experiments it was lower than during the first 5 minutes of action of the light.

In a series of experiments,lengthening the period of light stimulation to 30 minutes led to a further fall in the hyaluronidase activity.

To explain this phenomenon it was necessary to determine the antihyaluronidase factor in the nutrient solutions. For this purpose solutions were used which had contained preparations from rabbits' eyes exposed for 20 minutes to the action of light, or in which the preparations had spent only the last 10 minutes of a total exposure of 20 minutes to the light (Table 2).

It can be seen from Table 2 that antihyaluronidase activity was present in the nutrient solution containing the illuminated eye preparation, and as investigation showed, this factor was thermolabile.

In a series of experiments the activity of hyaluronidase and antifactor was determined several times in succession in the course of 20 minutes. The results of two of these experiments are shown in Table 3.

The gradual fall in the hyaluronidase activity of the nutrient solution in response to interrupted exposure of the retina to light for 20-30 minutes was not connected with loss of viability. This was shown by experiments in which light and dark were alternated every 5 minutes in the course of 20 minutes, in which corresponding fluctuations of hyaluronidase activity took place: light increased the hyaluronidase activity of the retina, but darkness reduced it. The level of activity of the hyaluronidase reaction in response to illumination remained within the usual limits (see Table 1).

The gradual fall in hyaluronidase activity of the nutrient medium during continuous exposure to light may be explained by the secretion by the retina of an antihyaluronidase factor, which we have discovered. The concentration of antifactor in the nutrient solution in which the surviving preparations of animals' eyes were kept underwent regular changes.

This antifactor is capable of inactivating hyaluronidase, by destroying it or by combining with it to form an inactive complex. The fall in the concentration of antifactor as well as enzyme suggests that they do combine together to form an inactive complex.

From the results obtained it may be postulated that the retina possesses hyaluronidase and antihyaluronidase activity. The hyaluronidase activity of the retina of animals depends on the intensity of the light stimulus and fluctuates regularly, being reduced in response to prolonged and continuous illumination. The antihyaluronidase factor has the power to inactivate hyaluronidase by combining with it to form an inactive complex.

SUMMARY

The retina possesses hyaluronidasic and antihyaluronidasic activity which depends on the strength of the light stimulus. In prolonged continuous light stimulation the retinal hyaluronidasic activity is diminished. The dynamics of decrease of the hyaluronidasic activity depends on its initial value: the higher the initial activity, the quicker its decrease.

The activity of the antihyaluronidasic factor, as well as the activity of hyaluronidase gradually decreases in continuous action of the light. The antifactor promotes the inactivation of the hyaluronidase combining with it into an inactive complex.

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THE EFFECT OF STATIC EXERCISE ON THE RATE OF CONTRACTION OF THE HEART

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Studies of the effect of static work on the rhythm of the heart have shown that this is accelerated [1, 2, 3, 6, 7]. Data on the rate of the blood flow [1, 8] and on the minute volume [4], however, indirectly show that there is depression of cardiac activity during static exercises.

The conflicting nature of these findings may possibly be explained by the fact that the static exercises studied in the research cited above differed in their character and difficulty.

In the present investigation we studied the effect of static exercises on the activity of the heart by means of static poses used in sport.

EXPERIMENTAL METHOD

The research was carried out with the aid of 17 weight lifters and gymnasts who performed various exercises. We confined our choice to static exercises used in sport because they permitted the activity of the heart to be studied in the same persons carrying out exercises of varying degrees of difficulty. Some of these (horizontal handstand, lifting weights twice as heavy as the athletes themselves, and so on) could only be done by highly qualified athletes.

Static exercises in such forms of sport as gymnastics and weight lifting are widely practiced and their study is of great interest.

The recording of the contractions of the heart during the performance of the static exercises was carried out by means of a photocell fixed to the pinna of the subjects. The EMF of the cell was fed into the input of an amplifier and recorded on a paper tape.

The following static exercises were performed: standing on the shoulders and hands, balancing (resting on the outstretched hands, and supported on the elbows, "swallow" and sideways, on one foot), resting (lying, lying on the thighs and lying on the hands), "bridge", hanging from a crossbar, lunging, lifting weights of different sizes while sitting, standing and with outstretched hands.

During the course of one experiment each subject carried out 10-15 exercises lasting 5-30 seconds, with intervals of rest of 5-10 minutes.

EXPERIMENTAL RESULTS

The course of the changes in the heart rate before, during, and after performing static exercises of a gymnastic character showed certain variations.

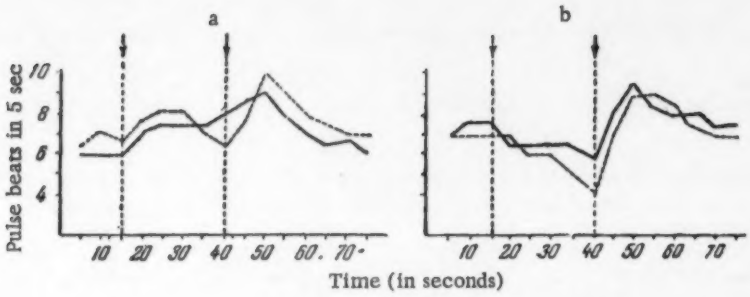

Fig. 1. Frequency of contraction of the heart before, during, and after the performance of static exercises (the times are indicated by arrows). a) ——— During performance of "angle at rest." Subject A, Grade 2 athlete; – – – – During performance of "balancing with elbow support." Subject T, Grade 1 athlete. b) ——— During the "bridge" exercise with forced breathing; – – – – the same, but while holding the breath. Subject Sh. Master of Sport.

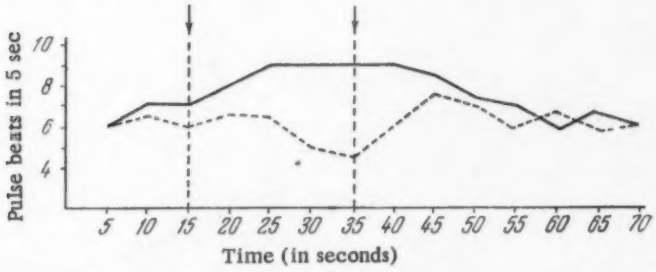

Fig. 2. Rate of the heart before, during, and after performance of static exercises (the times are indicated by arrows). ——— During lifting a weight of 100 kg, sitting, by the "scissors" method; – – – – During the exercise of "handstand." Subject P, Grade 2 weight lifter, welterweight.

In the performance of the simpler exercises the pulse quickened, and after they were finished it slowed. When more complicated exercises were carried out the pulse quickened both during the exercise and during the first 7-10 seconds after their completion, followed by a fall in the pulse rate (Fig. 1, a, angle at rest). This effect was similar to Lindhard's phenomenon [5]. Sometimes during the performance of the exercise, in the first 10-15 seconds the pulse quickened and then slowed (Fig. 1, a, balancing with elbow support). In some cases at the end of the exercise the pulse rate was below the original value, and after completion of the exercise a sharp rise took place in the pulse rate, followed by a fall. Finally, during the performance of some exercises the heart rate was diminished from start to finish (Fig. 1, b).

The effect of static exercise was greatest in the last two variants. Such reactions were found in the performance of the most complicated exercises.

The effect of static exercise was observed in gymnasts, whatever their qualifications, and no difference was found during the performance of the same exercises by trained and untrained gymnasts. In gymnastics, training in static exercises did not, therefore, completely remove this phenomenon, and probably did not even reduce its degree of expression.

In static poses in which the athlete held his head down ("bridge", handstand), the effect of static exercise

was more pronounced than when the head was held upright (angle, lunging) or in the horizontal position (balancing).

During the performance of the same exercises the effect of static exercise was more pronounced in women than in men.

Thus in the performance of simple gymnastic exercises no effect was found from static exercise on the rate of the heart.

The performance of complicated exercises was accompanied by a reaction similar to Lindhard's phenomenon, and its intensity depended on the difficulty of the exercise and the character of the pose.

From the results which we obtained we considered that the effect of static exercise on the rate of the heart would be more marked in weight lifting. Experiments showed, however, that the changes in the rate of the heart during lifting of weights of different sizes were no different from those taking place during dynamic exercise. During the performance of gymnastic exercises by these weight lifters, an effect of static exercise was found (Fig. 2).

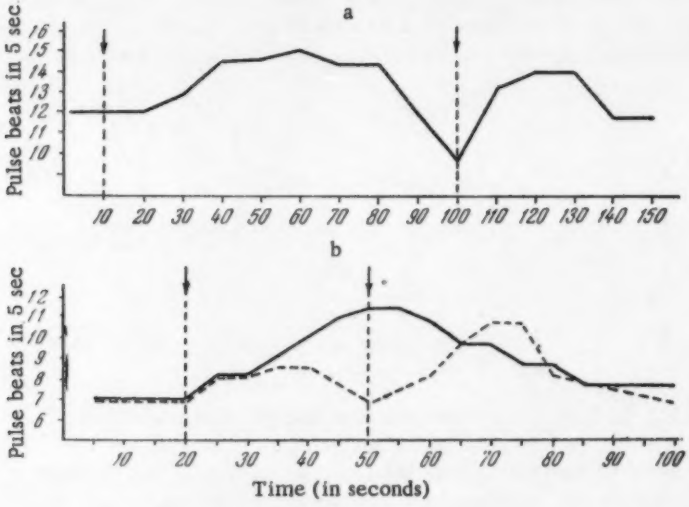

Fig. 3. The effect of holding the breath on the rate of the heart.
a) Heart rate while holding the breath at inspiration of 80% of the vital capacity of the lungs while sitting at rest.
b)—— during work on the bicycle ergometer; – – – – the same, but while holding the breath at inspiration of 80% of the vital capacity of the lungs. Uniform rate of work, 642 kgm in 30 seconds, rate 80 per minute; amount of work the same in both cases (times of working are indicated by arrows).

The effect of static exercise on the rate of the heart consequently depended on the character of the static pose.

The performance of static exercises is often accompanied by holding the breath and by straining. In special experiments during the study of the variation in the rate of the heart during and after holding the breath a phenomenon was found which was equivalent to that during static exercise (Fig. 3, a). It appeared only during great voluntary effort. In these cases a fall in the heart rate was sometimes observed to half the original value. If the subject was unable to hold his breath for a "limiting" period, this phenomenon did not arise.

We considered that the effect of static exercise on the activity of the heart was due to holding the breath. However, when the same exercises were carried out by the same persons during forced respiration or holding the

breath, it was found that an effect of static exercise was observed in both cases (Fig. 1, b).

Furthermore, in weight lifting with the breath held, and also during dynamic work with the breath held, a phenomenon was observed which was similar to that seen in static exercise (Fig. 3, b).

Thus while holding the breath (we are speaking here of holding it for a relatively long time), a phenomenon similar to that of Lindhard affecting the activity of the heart was observed both at rest and during static and dynamic exercise. Forced breathing during static exercise may minmize this phenomenon to some extent but it does not remove it completely.

In a study of the minute volume of the heart and the oxygen demand during static exercise, some workers observed Lindhard's phenomenon, others did not. We also observed that this phenomenon was sometimes present, sometimes absent. This fact, and also the undoubted resemblance of the changes in the heart rate, along with the characteristic changes in the minute volume and oxygen demand during static exercise, suggest that these phenomena have a common central mechanism.

It may also be postulated that the reflex which inhibits the cardiac activity during a static pose is one of the central links of the physiological mechanism of Lindhard's phenomenon.

The operation of this reflex causes corresponding changes in the minute volume and oxygen demand, and so possibly explains why in some cases Lindhard's phenomenon is observed during static poses and sometimes it is not. This hypothesis, however, requires special experimental verification.

SUMMARY

Cardiac contractions registered in 17 qualified gymnasts and weight lifters by means of a photoindicator and a phenomenon similar to that of "Lindhard" revealed that after cessation of the exercises, especially difficult ones, the rate of cardiac contractions increased sharply. This phenomenon was more pronounced in women than in men. There was no significant difference between qualified and nonqualified sportsmen.

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THE INOTROPIC AND CHRONOTROPIC COMPONENTS OF THE CARDIAC ACTIVITY OF DOGS AT REST

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Clinically there are distinguished [2] a "basal" or "residual" intra-arterial pressure, which is the lowest level obtained in repeated measurements in the absence of digestion and physical effort, and an "active", "chance" or "ordinary" pressure, obtained by the first measurement during a period of activity by the subject examined. Under these circumstances the transition from the "ordinary" to the "basal" pressure lasts 5-10 minutes.

In research carried out on dogs [6] it was shown that the values of the intra-arterial pressure fall if taken every day, reaching their lowest level in the course of a week. In some analogous investigations [11, 12] it was found that the process of this fall in pressure may last for several months. These authors, who experimented on dogs, were not concerned with determining the course of the changes in these values in the course of an isolated experiment. Nevertheless this is important in order to find out the mechanism of nervous influence on the heart when the animal is recovering in a state of rest.

Our aim was to discover the quantitative and qualitative features of the changes in the inotropic and chronotropic components of the cardiac activity of dogs during the period of transition from ordinary activity to the resting state, when kept in the laboratory for the course of the experiment. In carrying out this task it was necessary to determine the strength of the cardiac contractions by a method of bloodless examination. Among the various parameters of the variations in the intra-arterial pressure, the one that most often satisfies the requirements of such an investigation is the magnitude of the final maximum pressure (FMP). The basis of this choice is the idea [9] that the FMP reflects the whole potential and kinetic energy of the blood moving along the vessel at the point of measurement. In view of the fact that in the higher vertebrates the main source of energy of movement of the arterial blood is the heart, the FMP may act as an index of the energy developed by the heart muscle in the process of contraction. This is in accordance with clinical observations [1, 5, 7 and others], showing that the FMP reflects the work of the heart and the strength of its contractions.

EXPERIMENTAL METHOD

The experiments were performed on dogs in an ordinary experimental chamber, not screened from extraneous sounds. During the experiment the animal lay on the floor or in a cradle in a convenient position. The FMP was determined in the "intermammillary" vessels or in the central caudal artery every 1-2 minutes throughout the experiment, by means of a mechanocardio-oscillograph [4]. In each experiment from 15 to 50 measurements were made. At the same time continuous sphygmography was carried out, the results of which were used to ascertain the average frequency of cardiac contractions (FCC) and as an indicator of the degree of compression of the vessel during the determination of the FMP. The experiments were discontinued if the animal passed into a state of prolonged excitation. The FMP was estimated in mm of mercury.

EXPERIMENTAL RESULTS

Altogether we carried out 122 experiments on 4 dogs. It was found that in the conditions described above, the value of the FMP and FCC in these dogs gradually fell. This can be seen from the example of the experiment (with the dog Naida) of which the results are shown in Fig. 1; in the course of 25 minutes the value of the FMP fell by 35 mm and that of the FCC by 27 per minute. We then deduced the mean values each minute of the chronotropic and inotropic indices for all the experiments carried out with each dog. The mean values obtained were treated graphically in two ways. In the first variant values were not excluded which coincided with the action of extraneous sound stimuli; in the second variant the calculation of the mean values was preceded by exclusion of those results which coincided with these stimuli, until the values had returned to their initial level. In Fig. 2 are shown the results of 22 experiments carried out on the dog Trezor. It can be seen from this figure that the combined results for all the experiments also reflected the fall in the values of the FMP and FCC. We performed analogous experiments on 3 more dogs with the same results. In one of these dogs — Laski — a few years before the experiments the spinal cord had been divided in the lower thoracic region, and the abdominal sympathetic chains had been removed. We give a table which summarizes the results of the experiments on all the dogs which were studied.

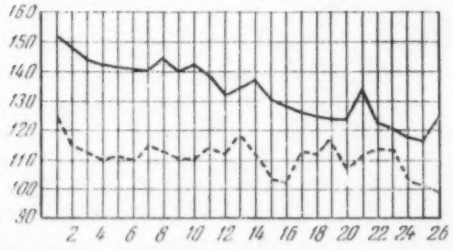

Fig. 1 Changes in the value of the final maximum pressure (FMP) and the frequency of cardiac contraction (FCC) in the dog Naida at rest.
Legend: along the ordinate axis — values of the FMP (in mm of mercury) and the FCC (per minute); along the abscissa — duration of the experiment (in minutes); ——— FMP, – – – – FCC.

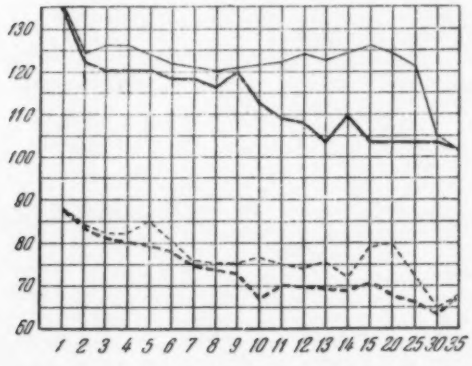

Fig. 2. The FMP and FCC of the dog Trezor at rest.
Legend: thin lines — first variant, thick lines — second variant (for explanation see text); remaining legend as in Fig. 1.

The experimental results showed that when the dog lay at rest there was a fall in the value of the FMP, as is also observed in human subjects on repeated examination in a state of rest. The duration of this process in animals reached 45 minutes in individual cases, compared with 5-10 minutes in man. On the average for all the experiments, in 35 minutes the FMP fell by 27.5 mm and the FCC by 26.5 per minute (second variant). There were two possible explanations of this phenomenon. The first was derived from the standpoint of the general functions of the circulation of the blood as an apparatus responsible for the adaptability of the body toward changed conditions of existence and maintaining the constancy of the internal environment of the body at the most favorable level for its vital activity [8]. According to this view, the fall which we observed in the inotropic and chronotropic indices could be regarded as the manifestation of a lowering of the general physiological activity of the dogs in the process of rest. The second was based on the hypothesis that when the animal was at rest the vascular tone was diminished, which was also the cause of the fall in the values of the FMP. Edens [3], who observed a fall in the blood pressure during repeated measurements in the course of 4-5 minutes, provisionally explained this as due to a fall in the resistance of the vessel wall under the action of compression in the process of measurement. This explanation, so simple and logical at first sight, conflicts with certain experimental findings.

Artificial increase of the tone of the vessel wall due to the action of adrenaline leads to a fall, but a decrease of the tone (by cocaine) leads to a rise in the external pressure required for compressing the vessel [10]. These experiments were in agreement with the clinical findings (angiospastic states) which suggested that an increase in vascular tone may cause intravascular occlusion of varying degree without the addition of an external compressing agent. This seemed to indicate that the direction of the

force during artificial or natural increase in the tone of the vessel wall coincides with the direction of the compressing agent during the determination of the FMP.

Changes in the Inotropic and Chronotropic Indices of Cardiac Activity of an Animal in a State of Rest (the dog in recumbency)

Name of dog	Number of experiments	Vessels used in the experiments	At the beginning of the experiments		At the end of the experiments		Fall in the values in the course of the experiments		Duration of the experiments, in minutes
			FMP	FCC	FMP	FCC	FMP	FCC	
Rozka	15	Intermammillary	116	126	106	95	10	31	30
Laska	20	The same	121	98	85	76	36	22	30
Trezor	22	Caudal artery	134	88	103	64	31	24	35
Naida	55	The same	139	102	106	73	33	29	45
Average	112	—	127,5	103,5	100	77	27,5	26,5	35

In connection with the above it became evident that the reduction in the tone of the vessels could not be the cause of the fall in the FMP if we were limited to a consideration of the factor described above. Experiments carried out on the dog Laska, in which the FMP was measured in vessels deprived of central nervous tonic influences from the brain, showed that a fall took place in the FMP as it did in the intact animals. If the cause of the fall in the FMP which we observed was a corresponding fall in tonic influences, then in the conditions of the experiments on this dog, the FMP would not have fallen when the dog was at rest. The fall in the above-mentioned values in this animal could be attributed to humoral factors, but then it would have to be accepted that these factors acted in isolation from the nervous influences, which is incorrect. It follows from the foregoing that the fall in the FMP and FCC which we observed in dogs in the resting state was a manifestation of changes in the adaptational-trophic influences acting on the heart.

The experimental findings which we obtained justify the conclusion that a state of rest (recumbency) in dogs in an ordinary experimental chamber is accompanied by intensification of negative inotropic and chronotropic influences on the activity of the heart. These changes, observed in experiments lasting up to 45 minutes, were present in both intact dogs and an animal in which the vessels at the point of measurement were deprived of tonic nerve influences from the brain.

SUMMARY

The values of the final maximal intra-arterial blood pressure and the average frequency of cardiac contractions were studied in 4 dogs which lay at rest. In experiments of up to 50 minutes there occurred a gradual reduction of these values depending on the duration of the rest period. This regularity was observed both in dogs intact and in animals whose vessels were not subject to the cerebral neural tonic effect at the site of examination.

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THE EFFECT OF STIMULATION OF THE MECHANO–AND CHEMORECEPTORS OF THE ORAL CAVITY ON CARBOHYDRATE METABOLISM

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In previous communications devoted to the study of the effect of stimulation of the receptors of the oral cavity on metabolism, the effect of the duration of stimulation on the taste receptors of the mucosa of the oral cavity on the regulation of carbohydrate metabolism was established. It was shown that prolonged sucking (for one hour) of 15 g of candy drops causes an intensive increase in the blood sugar and in the oxygen demand, which continues for many hours. If the same quantity of candy drops were eaten quickly, only a transient rise in the carbohydrate metabolism was produced [1, 2].

It is well known that in the higher animals and in man the mucous membrane of the oral cavity not only possesses taste receptors but also contains a very complex arrangement of mechanical, chemical and temperature receptors.

The present investigation was devoted to the further study of the influence of stimulation of the receptors of the oral cavity on the level of the blood sugar and on the gas exchange. It appeared necessary, in the first place, to study the influence of sucking substances of no food value on the regulation of carbohydrate metabolism and thereby to ascertain the role of the mechanoreceptors of the oral cavity in metabolism, and in the second place to define the conditions in which subthreshold concentrations of chemical (taste) stimuli become threshold stimuli, perceptible to the taste and having an effect on the regulation of carbohydrate metabolism.

EXPERIMENTAL METHOD

Observations were made on 6 healthy human subjects, ages from 18 to 49 years. As a chemical (taste) stimulus glucose was used, a 4% solution having hardly any sweet taste. The mechanical stimulus was sucking a round glass disk, which resembled a candy drop, being 3 mm in thickness and 2 cm in diameter. The disk was kept in the subject's mouth for 1 hour without causing any sensation of taste. For greater convenience the glucose was poured into a burette, from which it passed through a rubber tube and a mouthpiece, to emerge into the subject's mouth in drops, where it was gradually swallowed.

We performed several series of experiments in order to study:

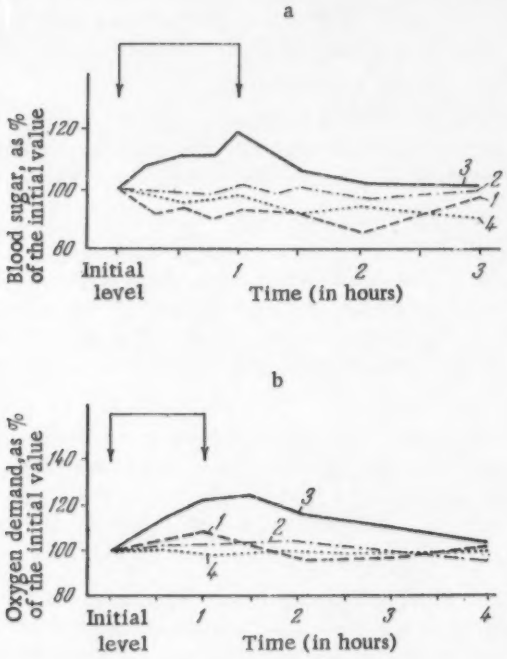
1) the effect of sucking the glass disk for 1 hour on the level of the blood sugar and the gas exchange;

2) the effect of introducing 100 ml of 4% glucose solution, in drops in the course of 1 hour, into the subject's mouth, on the level of carbohydrate metabolism;

3) the effect of administration of 100 ml of 4% glucose solution in the course of 1 hour to the subject by mouth, with simultaneous sucking of the glass disk, on the blood sugar level and the gas exchange.

We also carried out preliminary control experiments for a period of 3 hours to investigate the blood sugar level and the gas exchange. Altogether 24 experiments were performed, and the results of each series were in agreement. All experiments were carried out in the morning, on fasting subjects, within a period of 3 hours.

The blood sugar concentration was estimated by the Hagedorn-Jensen method, before the application of any form of stimulus, and then every 15 minutes during the hour that the stimuli were in action, and subsequently after 30, 60 and 120 minutes. The gas exchange was measured by the Zuntz-Haldane method, before application of the stimuli, and then over periods of 10 minutes every 30 minutes throughout the period of the observations.



The effect of stimulation of the receptors of the oral cavity on the level of the blood sugar (a) and the value of the oxygen demand (b).
1) Sucking a glass disk; 2) ingestion of 100 ml of 4% glucose solution in the course of 1 hour; 3) simultaneous ingestion of 100 ml of 4% glucose solution and sucking a glass disk; 4) control (mean values of all experiments). Duration of action of the stimulus one hour.

EXPERIMENTAL RESULTS

The results of the control experiments, carried out in the absence of any form of stimulus, showed (see Figure) that both the blood sugar and the gas exchange remained practically unchanged during the 3 hours of the experiment.

The study of the effect of sucking the glass disk on the blood sugar level and the gas exchange showed that if the disk was sucked for a long time it caused a gradual fall in the blood sugar level, which varied only very slightly from the fluctuations of the blood sugar level in the control experiments (see Figure, a). At the same

time there was a very insignificant rise in the gas exchange (by 4-8 %), and this was observed only in the course of the process of sucking itself, after which the level of gas exchange returned to its initial value as estimated before sucking the disk (see Figure, b).

These findings fully justified the view that sucking a glass disk – an object possessing no gustatory qualities – caused no perceptible change in the level of the blood sugar and gas exchange by comparison with the control results. It could therefore be postulated that mechanical stimulation of the receptors of the oral cavity during the act of sucking had no essential effect on the carbohydrate metabolism.

The results of the investigation of the effect of administration of 100 ml of 4% glucose solution on the carbohydrate metabolism are also shown in the Figure. In these experiments we studied the role of the taste receptors in the regulation of the carbohydrate metabolism, since the glucose solution entered the subject's mouth in drops and was gradually swallowed, so that mechanical stimulation of the receptors of the oral cavity by this means was avoided.

The observations showed that during the slow introduction of glucose solution into the mouth the blood sugar level and the oxygen demand did not differ essentially from their level in the control experiments. It had to be assumed that a 4% solution of glucose is a subthreshold stimulus and does not cause changes in the carbohydrate metabolism.

In the next series of observations the combined action of stimulation of the taste (chemo-) receptors and the mechanoreceptors on carbohydrate metabolism was studied. For a period of 1 hour the subjects sucked the glass disk, and at the same time received 100 ml of 4% glucose solution by mouth, in drops, through the mouthpiece. It was found (see Figure) that the simultaneous sucking of a glass disk and ingestion of glucose solution caused a marked rise in the blood sugar concentration, which was still above the initial level at the end of the 3 hours' observation.

The blood sugar level rose only 15 minutes after the beginning of the experiment, and after 1 hour it reached its highest value by comparison with the initial level (116.8%). In the next 2 hours the blood sugar level gradually fell, but nevertheless remained slightly above the initial value. Under these experimental conditions the gas exchange was also significantly increased throughout the period of observation. Thus by the combined action of glucose solution and sucking a glass disk the blood sugar and gas exchange exceeded the initial level throughout.

We may reach the following conclusion from our results: whereas stimulation of the chemo- or mechanoreceptors of the oral cavity, in isolation from each other, have no perceptible effect on the blood sugar level and the oxygen demand by comparison with the control findings, the simultaneous application of these stimuli leads to a prolonged increase of the blood sugar concentration and of the gas exchange. It must be pointed out that the subjects noticed the sweet taste of the glucose only when it was given in conjunction with the glass disk to suck, in the form of a 4% solution. It may, therefore, be considered that subthreshold stimuli of the gustatory nerves, giving rise to no sensation of taste and having no objective effect on the level of the carbohydrate metabolism, when applied at the same time as mechanical stimulation of the oral mucosa, do produce a sensation of taste and cause changes in the regulation of the carbohydrate metabolism. On the other hand, stimulation of the mechanoreceptors of the oral cavity does affect the regulation of carbohydrate metabolism, but only in the presence of a gustatory stimulus, albeit a subthreshold stimulus.

The results of our investigations confirm those of other authors. S. A. Kharitonov [5], for instance, found an increased sensation of sweetness during stimulation of the mechanoreceptors of the oral cavity. O. A. Naumova [3] demonstrated that chewing inedible material had no effect on the reaction of the vessels, whereas chewing tasty material led to changes in the vascular reactions depending on the character of the taste. During a study of conditioned vascular reflexes, A. T. Pshonik [4] experimentally converted a conditioned presensory exteroceptive vascular reflex into a conditioned reflex sensory vascular reaction, and by this means investigated the process of conversion of an imperceptible into a perceptible impulse.

On the basis of the findings in our experimental conditions it may evidently be stated that it is not only stimulation of the chemoreceptors but also stimulation of the mechanoreceptors in the mucous membrane of the mouth that is of importance in the act of sucking.

Thus the action of sucking, which is a complex combination of various influences of the food on the peripheral endings of the gustatory nerves, motivates the process of nervous regulation of carbohydrate metabolism in the intact animal.

SUMMARY

Experiments on 6 healthy young persons observed for 3 hours established that administration of 100 ml of a 4% glucose solution per os for one hour (chemical stimulation) caused no change in the levels of blood sugar and oxygen intake, as compared with controls. Neither did these indices change substantially after glass disks were sucked by the subjects for one hour (mechanical stimulation). However, when the two stimuli were applied simultaneously, the levels of the blood sugar and oxygen intake rose considerabley during the following three hours of observation. There appeared a sensation of sweetness which had been absent during drop administration of 4% glucose per os.

On the basis of this conclusion it was shown that stimulation, both of chemoreceptors and of mechanoreceptors in the oral cavity, plays a significant part in the act of sucking.

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THE SECRETORY FUNCTION OF THE SMALL INTESTINE IN DOGS AFTER RESECTION OF THE STOMACH

COMMUNICATION I. PERIODIC SECRETION

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The secretory activity of the small intestine after partial resection of the stomach has received inadequate study, in spite of its practical importance.

Of the few papers in the literature dealing with this subject, the majority are devoted to the study of the motor function of the small intestine. Investigations of its secretory activity have been mainly clinical. S. Ya. Mikhlin and L. M. Levitskii [4], for instance, studied the enzyme-secreting function of the small intestine in patients after resection of the stomach for cancer. They found that secretion of intestinal enzymes is increased after this operation, whereas the enzyme content of the pancreatic juice – trypsin and amylase – remains within normal limits and the lipase content is reduced.

A. A. Kardeeva [2] carried out similar investigations in patients after resection of the stomach for gastric ulcer. This author also observed an increase in the intestinal enzymes (enterokinase and phosphatase) after resection of the stomach.

Yu. K. Kvashin [3] observed a fall in certain pancreatic and intestinal enzymes (enterokinase, lipase and trypsin), and an increase in the phosphatase in patients after gastrectomy for cancer of the stomach, in the first few months after operation. At the end of the first year after gastrectomy the secretion of enterokinase and lipase, according to Kvashin's findings, increases and the trypsin content returns to normal.

Certain discrepancies in the results that were obtained were presumed to be due to the fact that none of the above-cited authors were able to examine pure intestinal or pancreatic juice.

Of the experimental work published recently, we are aware of only the researches of S. A. Selezneva [5] and A. I. Aivazyan [1].

The work of S. A. Selezneva was devoted to the study of the motor and secretory activity of the stomach and intestine after resection of various portions of the stomach. In examination of the secretion of the small intestine, only the total quantity of intestinal juice (the periodic secretion and the secretion in response to mechanical stimulation) and the residual solid content of the juice were determined. No changes in the secretion of intestinal juice after resection of the stomach were observed by this author.

A. I. Aivazyan [1] also found no essential changes in the secretion of intestinal juice after resection of $\frac{2}{3}$ of the stomach. No details of the enzyme content were given in this paper.

In connection with investigations carried out by a group of workers from the Laboratory of the Physiology and Pathology of Digestion on the mechanisms of compensation of the functions of the digestive apparatus after exclusion of various portions of the gastrointestinal tract, we made an experimental study of the secretory activity of the small intestine after partial resection of the stomach.

In the present communication we give the results of an investigation of the periodic secretion of the intestine after resection of two thirds of the stomach.

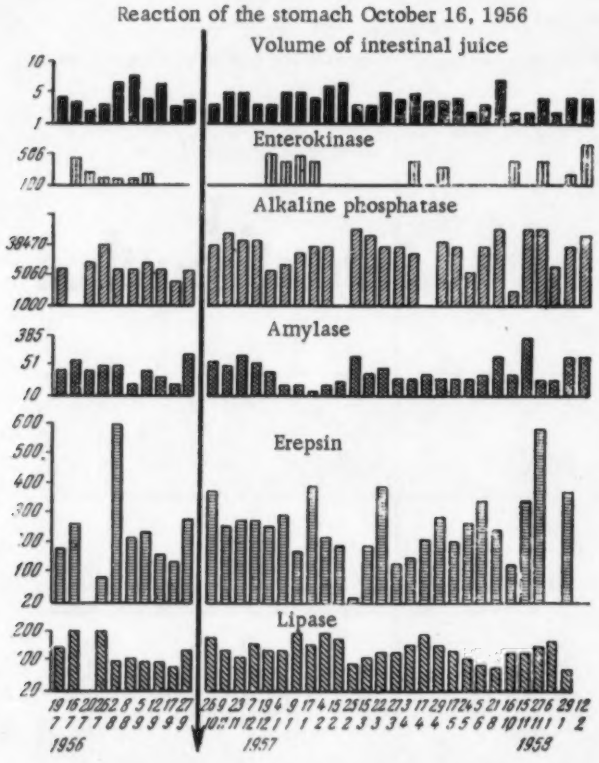

Fig. 1. Changes in the volume of intestinal juice and contained enzymes: enterokinase, alkaline phosphatase, amylase, erepsin and lipase in the dog Laima before and after resection of the stomach.
↓) Moment of resection of the stomach. Below, on the horizontal axis are given the dates of the experiments. On the left, on the vertical axis are given the quantities of the enzymes in conventional units.

EXPERIMENTAL METHOD

The investigations were carried out on dogs in which an isolated segment of the upper part of the small intestine had been fashioned by the Thiry method.

As indices of the secretory activity of the small intestine we selected the secretion of intestinal juice in an experimental period of 4 hours, the ratio of solids to fluid portion and the content of 5 enzymes in the juice: alkaline phosphatase, enterokinase, erepsin, lipase and amylase.

The proportions of solid and liquid parts were determined by centrifugation and subsequent resuspension.

The enzymes were estimated in a homogenate by methods devised or improved by the Institute of Nutrition of the AMN SSSR [6, 7].

The enzyme content was expressed as conventional units per ml of intestinal juice.

The results given in this communication were obtained with 4 dogs.

At the beginning of the investigations, in a series of experiments we estimated the content of all the above-mentioned components, after which a resection of the stomach was performed by the Hofmeister-Finsterer modification of the Billroth 2 method, and the investigations were then carried out 10-14 days later.

All the dogs, both before and after operation, were kept on a mixed, balanced diet. Food was given 2-3 times a day, in a finely chopped form.

The dogs were systematically weighed throughout the period of observation.

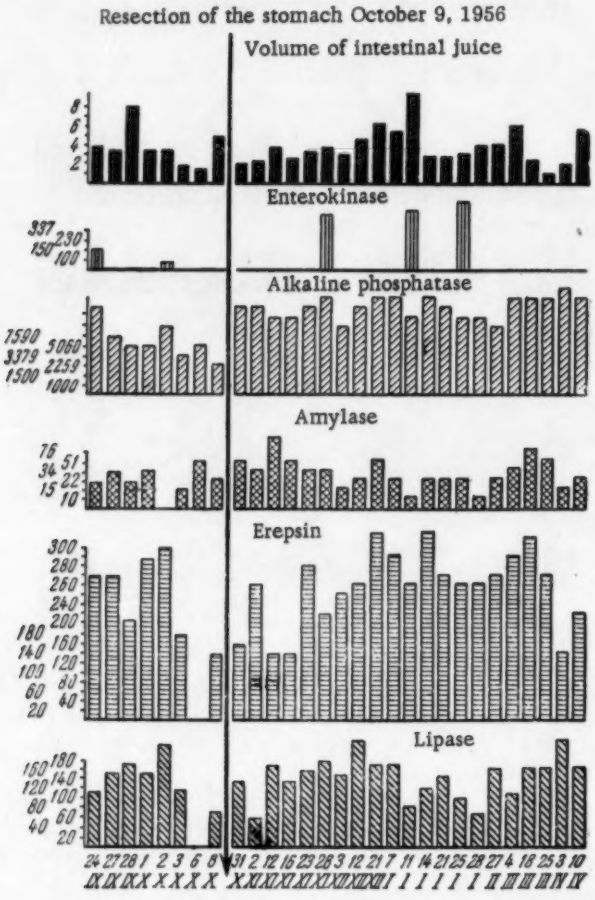

Fig. 2. Changes in the volume of intestinal juice and contained enzymes in the dog Dunai.

EXPERIMENTAL RESULTS

From the investigations which we made the following results were obtained.

The dog Laima was examined 1 year 4 months after resection of the stomach. The dog withstood the operation well. In the course of the first 2 months the weight of this dog fell by 2 kg, and it was then maintained at this level with slight variations on either side.

The results which we obtained during investigation of the secretion of intestinal juice in the dog Laima are shown in Fig. 1. The volume of intestinal juice obtained in the course of an experimental period of 4 hours did not change appreciably after resection of the stomach, i.e. the fluctuations which were observed from day to day after the operation did not exceed those which were present before the resection of the stomach. Nor did we observe any change in the proportions of solid and fluid parts of the juice.

In this particular dog, so far as the concentration of enxymes in the intestinal juice was concerned, we obtained a significant increase in the alkaline phosphatase, which began in the first month after resection of the stomach and has continued until the present time. The same feature was observed with regard to the enterokinase.

The concentration of erepsin and lipase also increased slightly. In the first 2 months after operation a slight increase in the amylase concentration could also be observed.

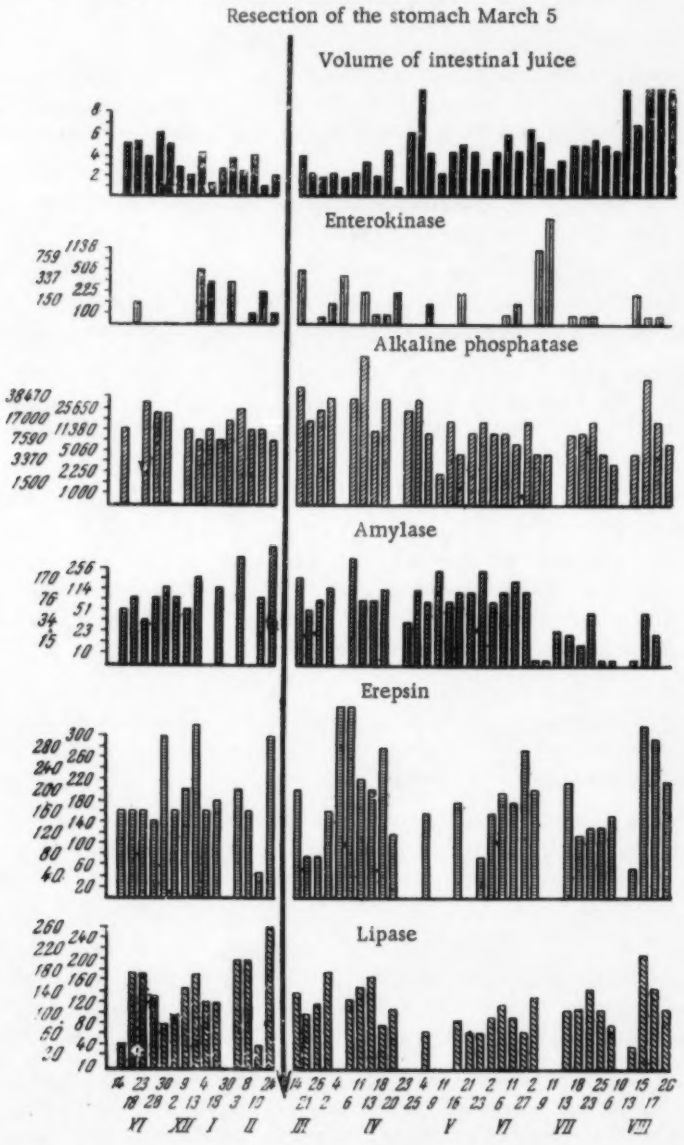

Fig. 3. Changes in the content of intestinal juice and its enzymes in the dog Mirta.

We also obtained similar results with the dog Dunai, which we were able to keep under observation for 8 months after operation. A significant increase in the concentration of phosphatase and enterokinase was observed, together with a small increase in the concentration of amylase in the first 2 months after the operation and, starting with the 2nd month, in that of erepsin. The variations in the concentration of lipase in the intestinal juice of the dog Dunai did not exceed those present before operation (Fig. 2).

In the third dog Anitra we also observed an increase in the concentration of phosphatase, but only in the first 2 months.

At later periods the variations in the phosphatase concentration did not exceed those before operation. A significant increase was observed in the amylase content, beginning with the 3rd month after resection of the stomach and continuing until the death of the dog. The content of the remaining enzymes investigated in the dog Anitra (lipase and erepsin) did not change significantly, according to our findings.

It must be pointed out that in the dog Anitra not two thirds of the stomach was resected, as in the other dogs, but four fifths. After the operation the dog lived for 12 months. The excitation of this dog towards food was sharply reduced. Beginning on the 8th month after resection of the stomach, the dog's weight began to fall progressively, and at the end of the 12th month, the dog died with manifestations of severe cachexia.

The fourth dog – Mirta – survived for 5½ months after resection of the stomach. From the first few days after the operation, the dog's weight fell progressively until the moment of its death. After operation jaundice was observed. The excitation of this dog towards food was reduced.

The volume of intestinal juice and its enzyme content were not appreciably altered, apart from a slight increase in the concentration of phosphatase in the course of the first month after operation (Fig. 3). During the 10 days before the dog's death, when its condition was grave, the volume of intestinal juice increased.

It can be concluded from the investigation that after partial resection of the stomach there is a tendency for the enzyme activity of the "periodic" juice of the small intestine to increase. The degree of increase of concentration of the enzymes after operation is expressed differently in respect of the various enzymes and the individual dogs.

The sharpest increase affected the phosphatase: in 3 of the 4 dogs examined, the increase in its concentration was significant and lasting (for 1 year 4 months); in the fourth dog – Mirta – an increase in the phosphatase was observed only in the first 1½ months. We observed an increase in the concentration of enterokinase in 2 of the 3 dogs examined (not in Mirta). We observed an increase in the concentration of erepsin after operation in 2 dogs; in the other two there was no change. The lipase concentration in the intestinal juice after removal of the stomach rose slightly in 2 dogs and in the other two its variations were with normal limits.

It may be pointed out that the greatest increase in enzyme activity of the intestinal juice was revealed in the dog whose general condition remained good for a long period of time after the operation (Laima). Conversely, in the dog Mirta, whose condition after resection of the stomach was serious, and which died 5½ months later with signs of cachexia, we found no appreciable changes.

It may be thought that the increase, after resection of the stomach, of mainly those enzymes of the intestinal juice which are concerned with the breakdown of protein is directly connected with the severe disturbance of digestion of proteins in the higher portions of the digestive tract, and is, therefore, compensatory in character.

SUMMARY

Data concerning the secretory and enzyme-secretory function of the small intestine after resection of ⅔ of the stomach in dogs was studied. The amount of the intestinal juice in periodical secretion, the ratio of the fluid to the solid part and the content of the 5 enzymes—enterokinase, basicphosphatase, erepsin, lipase and amylase—were examined.

It was demonstrated that there is a tendency to increased enzymatic activity of the intestinal juice after partial removal of the stomach. The most considerable increase was exhibited by phosphotase and enterokinase. The increase was more pronounced in the dogs, the general condition of which, after the operation, was good.

It is believed that the main increase of the activity occurring in the enzymes which take part in the protein

cleavage is in direct relationship with the acute disturbance of the process of protein digestion in the upper part of the digestive tract and is, therefore, compensatory in character.

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THE PARADOXICAL ACTION OF LARGE DOSES OF CORTISONE IN INFECTIVE AND ASEPTIC INFLAMMATION

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Cortisone is one of the most active anti-inflammatory preparations in clinical use at the present time. It is mainly given in hyperergic inflammation, for example in rheumatism. The administration of cortisone to patients with infectious conditions was considered to be contraindicated on the grounds that the drug lowered the resistance of the body. During recent years this view has been examined more closely and partially revised in connection with reports of the beneficial effects of small doses of cortisone in the treatment of a number of infectious diseases [1, 3, 6, etc.], and that the action of the drug depends on its dosage. The anti-inflammatory action of cortisone has been studied mainly in experimental aseptic inflammation; for this reason it is not certain to what extent the relationships found under these conditions are applicable to the more complex, infective inflammation. It is also uncertain to what general extent and with what consequences for the body the inflammatory reaction may be suppressed by the use of cortisone. In order to investigate these problems we studied the action of various doses of cortisone on inflammatory foci produced in white mice by subcutaneous inoculation of a culture of Friedländer's bacillus and of a solution of agar-agar.

EXPERIMENTAL METHOD

Mice weighing 14-16 g were inoculated subcutaneously in the right upper quadrant of the abdomen with a 20-hour culture of Bacillus pneumoniae suspended in physiological saline in proportion of 20-40 organisms to 0.04 ml of fluid. The virulence of the culture was maintained by daily passage through animals.

In another series of experiments, 0.04 ml of 0.008% solution of agar-agar in a 0.85% solution of NaCl was injected in the same region. 24 hours after injection of the culture of the microorganisms, and 4 hours after injection of the agar, the animals were killed and from the region of the injection were excised fragments of loose connective tissue, which were spread out on the surface of glass slides, fixed for 40-60 minutes in Shabadash No. 1 fixing agent* and then stained by Romanowsky's method. The number of the different cells in an area of 5.678 mm^2 of the stained films was counted by means of an ocular micrometer under the microscope with a magnification of 7 x 90. The areas of tissue selected for counting were either those with an accumulation of microorganisms (staining well by Romanowsky's method) or those with the most marked accumulation of polymorphonuclear leucocytes. The remaining fields of vision were selected from those in the immediate vicinity of the original.

The index of intensity of inflammation which we used was the ratio between the number of granulocytes and the total number of cells in the areas examined. In addition to this, in the experiments on infective inflammation, the number of organisms in the preparation was determined. The number of organisms in individual cases was enormous and could not be counted accurately.

We therefore counted their numbers within the following ranges: from 1 to 10, from 10 to 100 to 1000 and over 1000 in the preparation.

* Spiritus vini 100.0; $CuCO_3$ 1.8; $CaCO_3$ 0.9; neutral formalin 40% 10.0.

Cortisone acetate was injected under the skin of the dorsum in the form of a suspension in 0.85% NaCl solution. The last (or only) injection of cortisone was given 24 hours before giving the agar-agar or culture of microorganisms.

TABLE 1

Proportion of Leucocytes to Total Number of Cells in Films from Healthy Animals in Different Counts

Count	1	2	3
Proportion, %	5.58 ± 0.8	6.325 ± 1.1	5.83 ± 0.38

TABLE 2

The Effect of Cortisone on the Number of Leucocytes in an Inflammatory Focus during Infective Inflammation

Group	Dose of cortisone, mg/kg	Number of animals in group	Leucocyte content as a % of the control group	Index of reliability of the difference t	
				of the control group	of group No. 3
1	1.3 daily for 3 days	7	58.6	2.42	
2	4 once only	7	74.2	1.675	–
3	4 daily for 3 days	14	51.05	3.25	–
4	20 once only	7	85.0	0.77	1.83
5	20 daily for 3 days	21	117.1	0.961	3.73

Note: Leucocyte content of the control group of films – 24.8 ± 2.54% of the total number of cells, where n (number of animals) = 28.

TABLE 3

Number of Microorganisms in Films of the Connective Tissue from the Region of the Inflammation after Administration of Various Doses of Cortisone to the Animals

Dose of cortisone, mg/ kg	Number of animals in group	Number of microorganisms in the preparation			
		from 0 to 10	from 10 to 100	from 100 to 1000	over 1000
		number of animals			
None given (control)	28	20	3	3	2
1.3 daily for 3 days	7	5	0	1	1
4 once only	7	6	0	0	1
4 daily for 3 days	14	10	1	1	2
20 once only	7	3	2	2	0
20 daily for 3 days	21	7	4	4	6

TABLE 4

The Effect of Cortisone on the Number of Leucocytes in an Inflammatory Focus during Aseptic Inflammation

Group	Dose of cortisone, mg/ kg	Number of animals in the group	Number of leucocytes as % of control group	Index of significance of the difference t	
				of control group	of group No. 4
1	1.3 once only	22	87.0	1.973	–
2	1.3 daily for 3 days	9	73.0	2.94	–
3	4 once only	12	75.1	2.84	–
4	4 daily for 3 days	5	68.0	3.78	—
5	20 once only	10	113.9	1.635	2.68
6	20 daily for 3 days	15	82.72	1.925	1.3

Note: The number of leucocytes in the control group of films was 58.15 ± 2.8 % of the total number of cells, wher n = 34).

EXPERIMENTAL RESULTS

The reliability of the method of counting the number of cells in the films was tested as follows. Films of connective tissue were taken from 8 healthy mice by the method described above. Independent counts of the different cells were carried out on a given area of these films by three persons. It was found that the variations in the total number of cells in various areas of the films and in different animals were considerable, but the percentage of leucocytes in these areas was quite constant, as can be seen from Table 1.

The mean difference between the indices obtained by different persons in counts of the same preparations was: between the 1st and 2nd – 0.625, between the 1st and 3rd – 1.975 and between the 2nd and 3rd – 2.156.

The probability of the independence of the indices obtained in the 1st and 2nd counts, calculated by the Student distribution, was p = 0.558, and that of the 1st and 3rd counts, p = 0.927.

It could thus be considered that the number of leucocytes in the connective tissue films from normal animals was quite constant, and the individual characteristics of the experimenter in the selection of a place for the counting and in classifying the cells were of no essential importance.

The changes in the number of leucocytes in the inoculated mice are shown in Table 2. When this Table is examined it must be borne in mind that after subcutaneous injection of B. pneumoniae, irrespective of the virulence and number of the organisms injected, death of the animals did not begin to take place earlier than 30 hours after infection, and so none of the experimental animals died spontaneously.

As may be seen from Table 2, the single or repeated injection of cortisone acetate in a dose of 4-12 mg/ kg led to an obvious and statistically significant decrease in the infiltration of the focus of infection with leucocytes. Increasing the dose of cortisone to 20-60 mg/ kg not only did not cause any further decrease in infiltration but, on the contrary, this was even enhanced.

In order to explain this feature it was essential to take into account the characteristics of the change in the number of microorganisms in the focus. As may be seen in Table 3, when large doses of cortisone were given there was a considerable increase in the number of microorganisms developing from an initial number of several tens of bacilli injected. It might be assumed that an increase in the number of microorganisms in the region of infection intensified the degree ofstimulation. On the other hand, this increase in number was itself, evidently, the consequence of the reduced activity of factors of defense, which in ordinary conditions restrain the proliferation of microorganisms injected into the tissues.

In the aseptic inflammation experiments the main features of the relationship between the degree of infiltration and the dose of cortisone were the same as in the experiments using the culture of B. pneumoniae (Table

4). The anti-inflammatory effect was gradually intensified as the dose of cortisone was raised from 1.3 to 12 mg/ kg, but further increase of the dose of cortisone led to perceptible weakening of the degree of suppression of infiltration, the differences between the actions of doses of 12 and 20 mg/ kg being sufficiently significant.

The relationship thus observed was not, evidently, a special peculiarity of the anti-inflammatory properties of cortisone, but was due to peculiarities of the reaction of the body to this form of experimental inflammation. In any case, in joint observations with N. A. Demidova, using the same method, on the anti-inflammatory action of diazolin, obvious suppression of infiltration was observed in response to the administration of 10 mg/ kg, whereas this effect was completely absent when the drug was given in a dose of 25 mg/ kg.

Cortisone has a very complex and widespread action on the various systems of the body, associated with the development of defensive reactions. The mechanism of its action on inflammation is not completely clear. Dougherty and Schneebeli [2] observed accumulation of hydrocortisone in the fibroblasts, and pointed out the characteristic rounding of these cells in animals which had been given injections of cortisone and hydrocortisone, and the high resistance of fibroblasts of a spheroidal shape to the action of substances tending to destroy them. These authors were inclined to connect the anti-inflammatory properties of corticosteroids with this effect on the fibroblasts. On the other hand, the findings of Opsahl et al. [4], Winter and Flataker [8], and Seifter et al. [5], and our own observations showed that the functional properties of the basic substance were essentially modified by the action of cortisone. In parallel experiments we were able to show that a fall in the permeability of the basic substance to the spread of dyes and of bacteria could be observed after administration of small doses of cortisone, having an anti-inflammatory action. This effect on the basic substance ceased to be found in response to injection of doses of 20 mg/ kg and above. Thus the change from small to larger doses of cortisone was associated with a number of changes in the functional condition of the tissues.

Spain and Molomut [7] observed increased tissue necrosis in areas of turpentine inflammation in animals receiving large doses of cortisone.

From the above it may be assumed that in response to cortisone the nonspecific resistance of the tissues to injurious agents is reduced, and this reduction becomes considerable in degree when the dose of cortisone is increased. This is clearly demonstrated by the sharp rise in the number of Friedländer bacilli in the corresponding films of connective tissue. The great degree of tissue injury evidently acts as an additional stimulus to intensify the inflammatory reaction, possibly through mechanisms not concerned with the effect of cortisone.

This hypothesis requires further proof and verification. On the basis of the facts described, however, it may be considered that in any case the degree of suppression of the inflammatory reaction by cortisone is confined between definite limits. The attempt to increase the anti-inflammatory action by giving more than the optimum dose is not only ineffective but also dangerous, on account of the fall in the activity of the humoral and tissue defensive factors.

The changes taking place in the local inflammatory focus are evidently the result of changes in the general reactivity of the body under the influence of cortisone, although the actual mechanisms by which cortisone affects the systemic reactions of the body have been studied very inadequately.

SUMMARY

The author studied the effect of various doses of cortisone on the aspetic (agar-agar) and infectious (B. pneumoniae) inflammation by the method of determination of the degree of the leucocytic activity of the friable connective tissue. The quantitative regularities governing the depression rate of the infiltration by different doses of cortisone were found to be the same in both types of inflammation. It gradually rises with the increase of the cortisone dose from 1.3 to 12.0 mg/ kg. By increasing the cortisone dose to 20 mg/ kg the infiltration becomes still greater. The apparent absence of any effect of large cortisone doses on the inflammation is due (in infectious cases) to a considerable increase in the number of microbes at the site of their introduction and points to the decline in the tissue factor activity of the body resistance.

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THE MECHANISM OF DEVELOPMENT OF GAS GANGRENE

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The study of the general and special features of the development of a toxemic condition is of great importance in analysis of the mechanisms of the disease and in the search for methods of experimental therapy.

In recent years these problems have been the subject of systematic investigation in our laboratory. Particular attention has been devoted to the study of the role of reflex mechanisms in the process of development the course and the outcome of experimental gas gangrene [1-4]. As a result of these investigations the importance of pathogenic stimuli and the mechanisms which mediate them was revealed, both in relation to the disease and to the increase in the resistance of the body to the toxemic stimulus.

When studying the pathogenesis of gas gangrene we were confronted with the necessity of evaluating the role of two mechanisms in determining the development of the pathological process: the reflex and the "automatic" action of the toxins. It was necessary to take into consideration the fact that in the conditions of development of gas gangrene, besides pathogenic stimulation of the corresponding nerve endings in the affected focus, there also occurs a release of toxin into the internal media of the body, into the blood stream and the central nervous system. The question arose, what part did these mechanisms play in the general pathogenesis of the disease.

It had to be remembered that the degree of involvement of the reflex and the "automatic" components in the pathogenesis of the disease may differ and may vary depending on many factors, in particular on the stage of development of the infection.

In our previous research we observed that the reflex component of the disease plays an important part, especially in the first stages of development of the illness. In these stages it is possible, in the majority of cases, to prevent the development of the pathological process by measures directed toward changing the reactivity of the nervous system (novocain, interruption of the reflex arc in its afferent, efferent and central links, and so on). A later stages these measures, as has been shown experimentally, are incapable of arresting the development of the disease, evidently on account of the exclusion of mechanisms acting on the "automatic" principle.

The work of A. N. Aliev [1] showed that the pathogenetic importance of these two components may vary in accordance with the actual conditions of development of the toxemia. A subthreshold dose of the toxin of vibrion septique, injected directly into the substance of the cerebral hemispheres, produced a threshold effect only in association with the repeated injection of fractionated doses of the toxin intramuscularly. In other words, the preliminary summation of pathogenic stimuli, which in themselves did not cause toxemia, was able to increase to a considerable degree the reactivity of the central nervous system to toxin injected directly into the brain. When the same experiment was carried out, but with the order of carrying out the procedures changed, no effect was found on the course of development of the toxemia.

TABLE 1

The Effect of Decortication of White Rats on the Development of Toxemia after the Administration, by Various Methods, of Lethal Doses of Toxin of Vibrion Septique

Group No.	Experimental conditions	Animals	Number of animals in the experiment	Number of animals	
				dying	surviving
1	Injection of toxin into the brain	Experimental	23	21	2
		Control	23	16	7
2	Intravenous injection of toxin	Experimental	8	8	0
		Control	8	7	1
3	Fractionated injection of toxin into the limb muscles	Experimental	8	1	7
		Control	8	7	1

TABLE 2

The Effect of Changes in the Reactivity of the Central Nervous System on the Therapeutic Action of Specific Antiserum

Group No.	Experimental conditions	Number of animals in the experiment	Number of animals	
			dying	surviving
1	Intravenous injection of specific antiserum	19	10	9
2	Intravenous injection of specific antiserum in association with sodium amytal narcosis	24	6	18
3	Control	43	43	0

To continue our analysis of these two components of the mechanism of development of gas gangrene infection and toxemia, we attempted to find conditions in which their action could be disassociated within certain limits, and their importance in the pathogenesis of the disease individually assessed. In our experiments [5] it was found that after decortication of white rats, gas gangrene did not develop in response to inoculation of a lethal dose of a culture of vibrion septique into the muscles of the limbs; nor did toxemia develop in the animals after repeated fractional injection of the toxin of the same microorganisms into the muscles.

The question arose, did decortication affect the reactivity of the central nervous system only in relation to stimulation coming from the periphery, or could such a procedure alter the reactivity of the animal also in relation to toxin, acting according to the so-called automatic principle. In order to analyze this problem we carried out the experiments described below.

Three weeks before injection of the toxin, the cerebral cortex of white rats was extirpated in areas (frontal, parietal, temporal) accessible for operation. The animals undergoing operation were divided into 3 groups. Control animals were selected by weight to each group.

The first group of experimental and control animals was injected with toxin of vibrion septique (series 406) in a dose of 0.1 mg (2.5 MLD for mice) in the substance of the parietal region of the left cerebral hemisphere, the second group received a dose of 0.3 mg (7.5 MLD for mice) intravenously; and the third group of animals received a dose of 8 mg of toxin (200 MLD for mice), by fractionated injection of 10 MLD every 30 minutes, into the muscles of the hindlimb.

As may be seen from Table 1, the decorticated animals (with the exception of one rat) did not die after fractionated injection of the toxin into the muscles in doses causing the death of control animals (third group), whereas after injection of toxin into the brain of decorticated animals (first group) or into the blood stream (second group), the majority of them died from toxemia, just as did the control animals in these groups. Summation of pathogenic stimuli, taking place according to the reflex principle, was thus not observed after decortication, despite the fact that the reactivity of the animal to the automatic action of the toxin was not changed.

This showed that a change in the functional state of the central nervous system could entail dissociation of the reactions of the animal to the action of a toxin when administered by various methods. The consideration of this possibility and the discovery of the conditions in which this dissociation could be produced are of great importance in both the analysis of the pathogenesis of the infectious process and the study of the mechanisms by which the infection may be prevented.

We carried out a therapeutic experiment in gas gangrene, taking these findings into consideration.

When gas gangrene had already developed, and procedures directed towards removal of pathogenic stimuli were in themselves ineffective, and when specific forms of treatment (therapeutic serum) were also relatively ineffective, good results might have been expected from combined methods based on the above-mentioned features of the pathogenesis of toxemia in gas gangrene.

White rats (86 in number) were inoculated in the muscles of the hindlimbs with 0.1 ml of a suspension of vibrion septique organisms, diluted 1:1000, in a 5% solution of calcium chloride (prepared from a standard glycerol suspension of microorganisms containing 6×10^9 organisms per ml). The original suspension was diluted before the experiment with sterile physiological saline. 6 hours after inoculation the first group (19 rats) was injected intravenously with specific antiserum (0.1 ml 25 antitoxin units); the second group (24 rats) receive injections of serum after 6 hours during narcosis with sodium amytal, and the third group (43 rats) remained as controls.

The results of this experiment are shown in Table 2.

As may be seen from Table 2, the action of specific serum in association with sodium amytal narcosis (lowering the reactivity of the central nervous system to the pathogenic stimulus) had a favorable effect on the course of the disease.

Whereas the dose of serum chosen by itself could prevent death of only half the animals from gas gangrene, the same dose of serum in conjunction with sodium amytal narcosis was considerably more effective and preserved the lives of the majority of the animals. It must be remembered here that the animals were inoculated (as may be seen from the control group) with an absolute lethal dose of microorganisms.

A combination of pathogenetic measures, directed on the one hand toward decreasing the reactivity of the nervous system towards the action of pathogenic stimuli and on the other hand to the action of the specific stimulus itself, was found to be more effective than each of these procedures carried out separately. This conclusion became still more obvious when it was remembered that, by itself, sodium amytal has no essential effect on the disease at this stage of its development, and is able only to prolong the life of the animals. Nor does it have any effect, as shown by S. V. Magaeva's experiments, on the reactivity of the animal to vibrion septique toxin injected directly into the brain.

The consideration of both components of the pathogenesis of toxemia in gas gangrene thus enables more effective methods of combined treatment of this disease to be designed.

SUMMARY

It was possible to reveal the dissociation between the reflex and the "automatic" actions of the vibra on septique toxin in decorticated animals. In these conditions the white rats perished when the toxin was injected into the brain or blood. In repeated intramuscular injection of the toxin in fractional doses the process of summa-

tion of the pathogenic stimuli was absent and the animals did not die from intoxication.

The pathogenetic experimental therapy of gas gangrene was conducted with consideration of this regularity when coupled with the barbamyl sleep, which decreases the reactivity of the animals to the pathogenic stimulation. The specific serum gave a better effect, than the separate use of these components.

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NEURO-ENDOCRINE RELATIONSHIPS IN EXPERIMENTAL HYPERTHYROIDISM

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We know that the origin of the principal manifestations of hyperthyroidism is largely connected with increased activity of the sympathetico-adrenal system [6, 7].

In recent years evidence has accumulated of the beneficial effect of phenothiazine derivatives in hyperthyroidism [8, 9, 10]. These drugs possess marked sedative and adrenolytic properties. Their mechanism of action is associated with blocking of adrenergic processes in the substantia reticularis of the brainstem [7, 5] and also with the sympatholytic effect on the peripheral adrenergic structures [4].

The purpose of the present investigation was to study the action of one of the phenothiazine derivatives – aminazine (largactil) – on the state of certain somatovegetative functions in experimental hyperthyroidism.

EXPERIMENTAL METHOD

The investigations were carried out on 4 dogs. The sedative action of aminazine was studied by the changes in the behavior of the animals and the state of their conditioned reflex activity (by the motor defensive method with electrodermal reinforcement).

The adrenolytic effect of the drug was investigated by the changes in the pressor action of adrenalin, which was injected intravenously in a dose of 0.5 ml of a 1:40,000 solution, 20-25 minutes after the intravenous injection of aminazine.

The dose of aminazine used remained constant – 1 mg/ kg. In the conditioned reflex chamber the drug was injected 20 minutes before the experiment.

The action of aminazine on the circulation was studied outside the chamber from the changes in the arterial pressure and the ECG findings. Experimental hyperthyroidism was induced by feeding the dogs on thyroidin, in a dose of 0.5 g per kg body weight daily for 10 days. Experiments to study the sensitivity of the dogs to aminazine during administration of thyroid were carried out after an interval of 3-4 days.

EXPERIMENTAL RESULTS

In control experiments aminazine had an obvious sedative effect – soon after injection of the drug the dogs fell asleep, hung on their straps, and developed an unusual stereotype of movements; all the animals showed a pronounced enophthalmos.

A total or partial loss of positive conditioned reflexes was observed, with a lengthening of the latent periods, and diminution of the value of the motor and respiratory components (Fig. 1, a). In some experiments there was also observed a diminution of the unconditioned reactions appearing in association with electrical stimulation of the skin.

In this particular dose, aminazine produced a more or less hypotensive effect in all the dogs — from 5-10 minutes after injection of the drug the diastolic pressure in the various dogs fell by 10-33% and the systolic by 5-25% (in relation to the initial pressures, taken as 100). A more potent hypotensive effect was observed in the animals with a higher initial level of the arterial pressure.

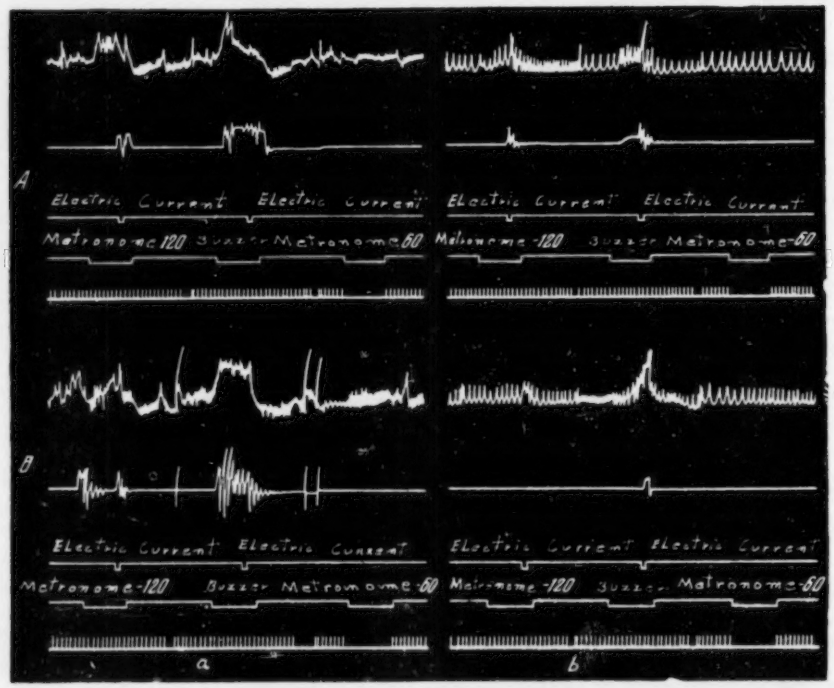

Fig. 1. The action of aminazine on the motor and respiratory components of the defensive conditioned reflexes during hyperthyroidism in the dog Belyi.
A) control experiments before injection of aminazine (a) and 20 minutes after injection of aminazine (b); B) hyperthyroidism: before injection of aminazine (a) (4th day of thyroidin administration), and 20 minutes after injection of aminazine (b) (10th day of feeding with thyroidin). Significance of the curves (from above down): respiration, motor reactions, marker of unconditioned stimulation with the electric current, marker of the conditioned stimuli, time marker (1 second).

In Tarzan, for instance, the arterial pressure fell 15 minutes after injection of aminazine from 170/120 to 130/80 mm of mercury, and in Belyi under the same conditions it fell from 130/80 to 120/70 mm of mercury.

The hypotensive effect of aminazin was accompanied by changes in the ECG findings — the R — R intervals were lengthened (bradycardia), the sinus (respiratory) arrhythmia was enhanced, as shown by the difference between $R-R_{Max}$ and $R-R_{Min}$, and also there was lengthening of the QRST complex (on the average by 0.03-0.05 seconds) and an increase in the positive potential of T_2.

The adrenolytic action of the drug was shown by diminution or distortion of the pressor reaction to adrenalin.

In the dog Ryzhii, for instance, adrenalin, given before the injection of aminazine, increased the arterial pressure from 150/110 to 200/150, 180/140 and 160/120 mm of mercury (at the 1st, 2nd and 3rd minutes respectively); after preliminary injection of aminazine the action of adrenalin in the 1st minute did not alter the arterial pressure, in the 2nd minute it fell (from 140/100 to 130/90 mm of mercury) and in the 3rd minute it regained its initial values (Fig. 2, a).

Experimental hyperthyroidism, caused by feeding the animals on thyroidin, was accompanied by considerable changes in these indices which we were studying.

The conditioned reflex activity of the dogs was altered after only the first dose of thyroidin, and moreover the character and degree of expression of these changes in the various dogs were different. In all 4 dogs, starting from the first days of administration of the hormone, the excitation of the respiratory component of the defensive reflexes was increased (the inspiratory dyspnea was intensified), the motor conditioned components were enhanced, and motor unrest appeared in the intervals between the stimuli.

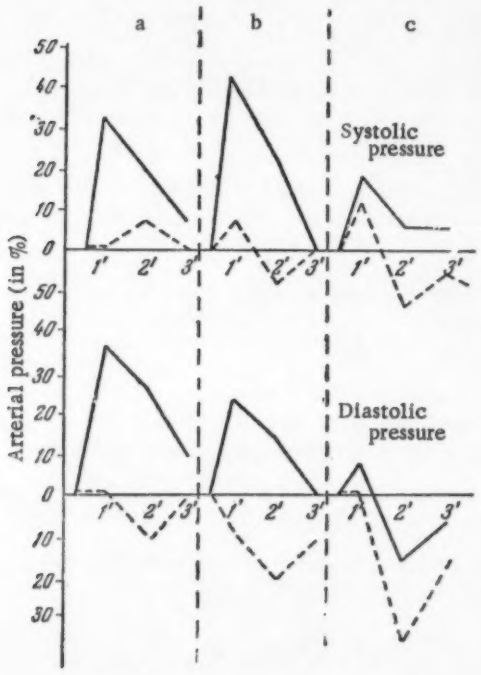

Fig. 2. The adrenolytic action of aminazine in association with hyperthyroidism in the dog Ryshii. Legend: ——— action of adrenalin; – – – – action of adrenalin after preliminary injection of aminazine; a) control; b) on the 3rd day of administration of thyroidin; c) on the 7th day of administration of thyroidin.

In animals showing strong excitation (Buyan and Tarzan) a shortening of the latent periods of the conditioned reflexed and frequent disinhibition of differentiation between motor and respiratory components were observed.

In animals with relatively weak excitation (Belyi and Ryzhii), thyroidin caused shortening of the latent periods in the first days of its administration, and on the following days it caused their lenghtening. Differentiation in these dogs was less often disinhibited, and mainly this affected the respiratory component.

From the 3rd day of administration of thyroidin, the arterial presure in all the dogs began to rise and the amplitude of the pulse increased; as the hyperthyroidism developed these phenomena increased.

In the dog Buyan, for instance, on the 7th day of administration of thyroidin the arterial pressure rose from 150/ 100 to 190/ 130 mm of mercury, and in Tarzan — from 170/ 120 to 200/ 140 mm of mercury. The increase in the arterial pressure and in the amplitude of the pulse were accompanied by marked changes in the ECG findings.

All the dogs showed considerable tachycardia, the pulse rate reaching 200 beats per minute, and under these circumstances the sinus arrhythmia diminished. In addition, in some animals there was shortening of the P — Q interval (by 0.03-0.04 second) and of the QRST complex (by 0.02-0.03 second) and increase in the negative potential of T_2.

Finally, the production of hyperthyroidism altered the sensitivity of the animals to adrenalin: its pressor action in the first days of feeding with thyroidin was enhanced, but later it decreased, and in some experiments adrenalin actually produced a perverted reaction, especially on the diastolic pressure.

In Tarzan, for instance, in control experiments, during the first minute of its action adrenalin increased the arterial pressure from 170/ 120 to 260/ 160 mm of mercury; on the third day of feeding with thyroidin — from 180/ 130 to 220/ 140 mm of mercury, and on the 7th day of administration of thyroidin adrenalin raised the systolic pressure from 200 to 240 mm of mercury and lowered the diastolic from 140 to 130 mm of mercury.

The induction of hyperthyroidism in the dogs thus caused a whole series of disturbances, the origin of which could be accounted for by changes in the excitation of the somatic and vegetative divisions of the central nervous system and by the peripheral action of thyroidin.

The enhanced respiratory components, associated with the action of the conditioned signals, the raised arterial pressure and the increased amplitude of the pulse, the tachycardia and the reduction of the sinus arrhythmia, and also the increased sensitivity of the animals to adrenalin — these all indicated the stimulation of the sympathetico-adrenal function.

In view of the findings of L. A. Orbeli [3], E. A. Asratyan [2] and other workers on the influence of the sympathetic division of the nervous system on the tonus of the cerebral hemispheres, it may be postulated that the change in conditioned reflex activity during hyperthyroidism was connected to a considerable degree with the increased activity of the sympathetico-adrenal system.

Tests of the pharmacodynamic properties of aminazine in dogs with hyperthyroidism have shown that all the effects of this drug observed in control experiments undergo considerable modification under these conditions.

The sedative action of aminazine appeared more intensively and was more prolonged in association with hyperthyroidism. Motor activity was suppressed to a greater degree than in control experiments; the animals remained for a longer time in a state resembling sleep.

Aminazine suppressed the motor and respiratory conditioned reactions more strongly. Under these circumstances there was also observed a weakening, even complete disappearance, of the unconditioned motor reactions connected with the afferent electrodermal stimulation (see Fig. 1, b).

The hypotensive effect of aminazine was more pronounced in association with hyperthyroidism, especially in the first days of thyroidin feeding: the arterial pressure fell after injection of the drug (the diastolic by 15-43%, the systolic by 7-37%).

In Tarzan, for instance, on the 3rd day of administration of thyroidin, aminazine lowered the arterial pressure 15 minutes after its injection from 170/120 to 110/70 mm of mercury, whereas in a control experiment it lowered it from 170/120 to 130/80 mm of mercury.

With the development of increasing hyperthyroidism in all the dogs, with the exception of one (Buyan), the hypotensive action of aminazine (expressed as a percentage) gradually decreased; in absolute figures the hypotensive effect was greater throughout the entire period of hyperthyroidism than in the control experiments.

The action of aminazine on the cardiac rhythm during hyperthyroidism was changed in the following manner.

In 3 dogs, especially in Ryzhii and Buyan, aminazine caused a lesser degree of bradycardia and not such a marked increase in the sinus arrhythmia as in control experiments.

In Ryzhii, for instance, in a control experiment the intervals, $R-R_{Max}$ and $R-R_{Min}$ were increased 15 minutes after injection of aminazine from 0.55-0.45 to 1.25-0.50 second, and on the 7th day of administration of thyroidin – from 0.45-0.40 to only 0.85-0.50 second.

In Belyi, in which initially the pulse was relatively slower and the sinus arrhythmia more marked, aminazine in association with hyperthyroidism caused a more intense bradycardia and sinus arrhythmia. The action of aminazine on the length of systole (QRST) and on the direction of the T_2 wave was fundamentally the same in hyperthyroidism as in the control experiments.

The adrenolytic action of aminazine in association with hyperthyroidism was shown more strongly in all the dogs. Under these conditions aminazine caused a more marked suppression and distortion of the pressor action of adrenalin, especially on the diastolic pressure.

For example, whereas in control experiments adrenalin combined with the administration of aminazine lowered the diastolic pressure of the dog Belyi in control experiments by 20 mm of mercury, on the 7th day of feeding with thyroidin, after the preliminary injection of aminazine, adrenalin lowered the diastolic pressure by 40 mm of mercury. The changes in the adrenolytic properties of aminazine during hyperthyroidism in the dog Ryzhii are shown in Fig. 2.

It can be seen from the results described above that the stimulation of sympathetico-adrenal function observed in association with hyperthyroidism was accompanied by increased sensitivity of the animals to the sedative, hypotensive and adrenolytic actions of aminazine.

On the pharmacological basis of the actions of the phenothiazine derivatives – the blocking effect on the adrenergic mechanisms of the substantia reticularis, and their sympathicolytic properties, it may be postulated that the stimulation of the sympathetico-adrenal function during hyperthyroidism and the origin of its chief manifestations were associated with the increase in the adrenergic activity of the substantia reticularis and with the change in the sensitivity of the peripheral adrenergic structures.

SUMMARY

The author studied the effect of aminazine (1 mg/ kg of body weght) on the somatovegetative functions in dogs with experimental hyperthyroidism induced by feeding them on thyroidin (0.5 gm/ kg). The intensification of the sympathetico-adrenal activity occurring in these conditions is associated with an increased sensitivity of the animals to the sedative, hypotensive and adrenolytic effect of aminazine. Basing upon the mechanism of aminazine action it becomes possible to explain certain manifestations of hyperthyroidism by the rise of the adrenergic activity of reticular formation and that of the sensitivity of peripheral adrenoreactive structures.

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EVIDENCE OF THE AUTOMATIC ACTION OF ISOHEMOTRANSFUSION ON THE FUNCTIONAL CONDITION OF THE NERVOUS SYSTEM

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In recent years a great deal of information has been obtained, showing the importance of changes in the functional condition of all links of the corticovisceral reflex arc in the mechanism of action of various forms of blood transfusion [1, 3, 4-11, 13, 18]. It has been demonstrated that various forms of blood transfusion modify the functional state of the central nervous system [1, 4, 11, 19, 20]. In this connection there is some immediate importance attached to the elucidation of the mechanisms by which the transfused blood changes the functional condition of the nervous system.

The role of reflex mechanisms in the action of the various forms of blood transfusion has been demonstrated by many workers [3, 6, 9, 11].

Bearing in mind the specific nature of isohemotransfusion it was to be expected that it would have the power to alter the functional condition of the nervous system automatically. This was suggested by N. A. Fedorov [18], but it has not been proved experimentally.

The proof of the automatic action of the transfused blood on the cerebral cortex has met with great technical difficulties. We know that blood transfusion modifies the conditioned reflex activity of the cerebral cortex [3, 6, 18, 20]. This could, however, depend on humoral factors. Strengthening the processes of inhibition in the cerebral cortex (by sodium amytal or bromide) alters the reaction of the body to the various forms of blood transfusion. This does not explain the mechanisms of its effect on the functional condition of the cerebral cortex. Finally, by intravenous injection of novocain before blood transfusion, the reaction of the body to the transfusion can be changed.

We carried out investigations on the superior cervical sympathetic ganglion which by its special features may be regarded as part of the central nervous system brought out to the periphery.

We started from the consideration that by denervation of the ganglion (by division of the preganglionic fibers) it is deprived of the possibility of reflex stimulation, so that the changes which could occur in the functional condition of the ganglion during blood transfusion could, under these conditions, take place automatically – by an "internal humoral route."

EXPERIMENTAL METHOD

The sympathetic nerve in cats under urethane anesthesia was dissected out and divided. An electrode was passed beneath the cranial end of the divided nerve. Stimulation, lasting 5 seconds, was carried out by means of an induction apparatus, with a current voltage of 2v and the distance between the primary and secondary induction coils 5-8 cm.

The blood pressure in the carotid artery was recorded on a kymograph by means of a manometer, and the contraction of the nictitating membrane by means of a lever which was attached to it.

The investigation was carried out on 27 cats, of which 17 were experimental and 10 were donors.

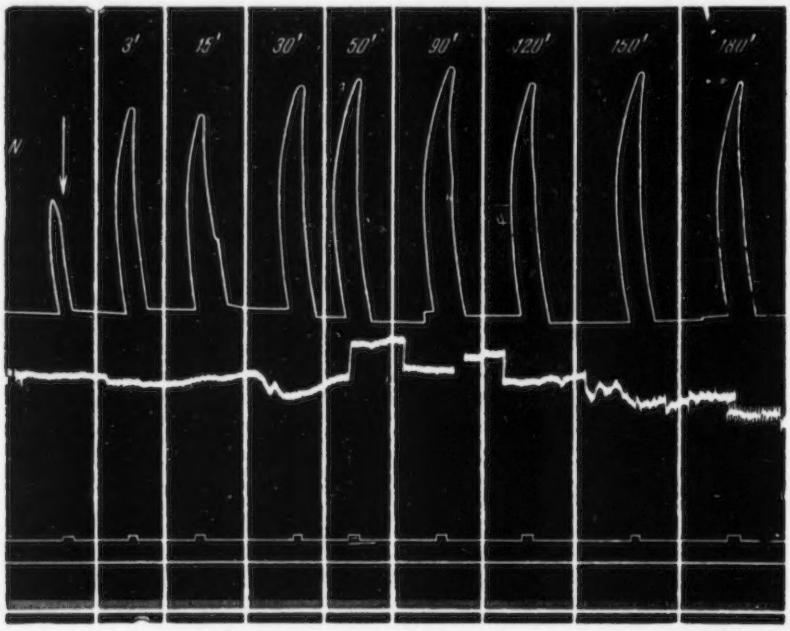

Fig. 1. The effect of blood transfusion on the functional condition of the superior cervical sympathetic ganglion of the cat.
Significance of the curves (from above down): contraction of the nictitating membrane, arterial pressure (mercury manometer), stimulus marker, zero line of the mercury manometer, time marker – 1 second; N – reaction of the nictitating membrane before blood transfusion; – isohemotransfusion – 4 ml/ kg. The figures above indicate the time in minutes after isohemotransfusion.

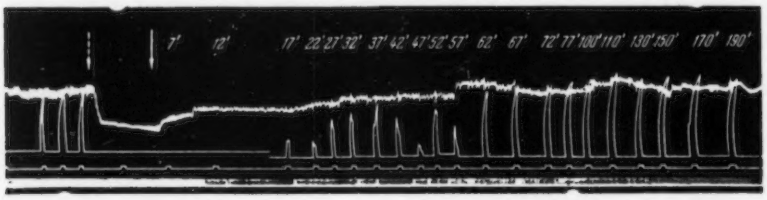

Fig. 2. The same as in Fig. 1, after preliminary administration of hexatonid. Significance of the curves (from above down): arterial pressure (mercury manometer), contraction of the nictitating membrane, stimulus marker, time marker – 1 second, zero line (of the mercury manometer); – injection of 10 mg/ kg of hexatonid; – isohemotransfusion.

EXPERIMENTAL RESULTS

In the first series of experiments (5 cats) we studied the effect of blood transfusion on the functional condition of the superior cervical sympathetic ganglion. The results of all the experiments followed the same pattern. Transfusion of blood (3-4 ml/ kg) caused a marked and prolonged increase in the tone of the nictitating membrane which was observed throughout the whole experiment (in individual cases up to 19 hours). This effect did not appear at once, but after the lapse of 3-5 minutes (Fig. 1).

Since the work was done on a specialized nerve-muscle preparation, the results could depend either on changes in the functional condition of the ganglion or on an increase in the reactivity of the effector organ (the muscle of the nictitating membrane). If the result obtained was due to a change in the functional condition of the ganglion, this effect would be absent after ganglion block.

In a second series of observations, made on 6 cats, a ganglion-blocking drug (10 mg/ kg of tetamon or the same dose of hexatonid) was first injected, and this was followed after 5 minutes by isohemotransfusion. The results after injection of both these drugs were in principle of the same pattern, but were more marked after hexatonid, which blocks the transmission of nerve impulses in ganglia more strongly and for a longer time (Fig. 2).

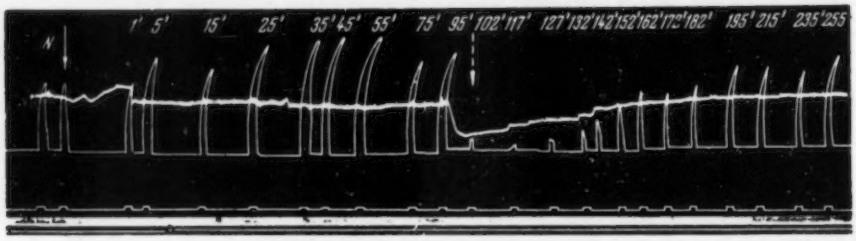

Fig. 3. The same as in Fig. 1, but hexatonid was given last. Legend as in Fig. 2.

The investigations showed that blocking the synaptic transmission in the sympathetic ganglion prevented the appearance of the effect due to isohemotransfusion. As the ganglion block died away, as shown by the rise in the arterial pressure, the effect due to isohemotransfusion reappeared.

In a third series of investigations (6 cats), the plan of the experiments was changed: isohemotransfusion preceded the injection of the ganglion-blocking drugs. As in the previous series, after injection of hexatonid and tetamon, results of the same pattern were obtained, but more marked after hexatonid. In the experiments of this series, transfusion of blood was accompanied by a pronounced increase in the tone of the nictitating membrane, which was removed during the 70-100 minutes of the blocking of the impulses in the ganglion. As the ganglion block wore off, the effect of isohemotransfusion was restored (Fig. 3).

Isohemotransfusion was thus accompanied in the cat by increased function of the superior cervical ganglion. Since these investigations took place after division of the preganglionic sympathetic fibers, the results proved that blood transfusion could act automatically on the functional state of the nervous system.

Information of importance in the solution of practical problems was forthcoming from our investigations.

In 1954 we proposed the use of ganglion-blocking durgs in the prophylaxis of transfusional reactions after various forms of blood transfusion. Clinical observations, which we made jointly with N. N. Novachenko [10], confirmed the value of this suggestion. The question naturally arose: did the administration of ganglionblocking drugs before transfusion prevent only the development of a pathological transfusional reaction or did it prevent the general reaction of the body to the blood transfusion. Clinical observations showed that the preliminary injection of ganglion-blocking drugs prevented the development of a pathological transfusional reaction without blocking the subsequent stimulating effect of the blood transfusion. The present investigations confirmed that the preliminary injection of ganglion-blocking drugs prevented the effects of isohemotransfusion only temporarily (for 70-100 minutes), after which they were shown in full measure.

The second practical problem consisted of the value of ganglion-blocking drugs in terminating already existing transfusional reactions. It was found that the administration of ganglion-blocking drugs after transfusion blocked a reaction which had developed to the transfused blood for a short time (70-110minutes).

The findings described, which indicate the possibility of an automatic action of transfused blood, thus provide a further experimental basis for the use of gangliolytic drugs in the prevention and treatment of transfusional reactions.

SUMMARY

Isohemotransfusion increases the function of a denervated sympathetic node (section of a preganglionic fiber) demonstrating the automatic effect of the transfused blood on the functional condition of the nervous system. The data obtained proves the expediency of gangliolytics administration for the prophylaxis and checking the transfusional reactions.

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SOME ASPECTS OF THERMOREGULATION DURING PREGNANCY

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Because of the successful use of drugs affecting the thermoregulation [aminazine (largactil) phenergan, etc.] and of methods of general hypothermia into the practice of obstetrics and gynecology, there is great interest in the problems of thermoregulation at the present time.

We know [11 and other] that two types of temperature curve may exist: the male, characterized by uniformity in time and with considerable diurnal variations (up to 0.9-1.0°), and the female, which is established at the time of puberty and which continues until the climacteric; the second type is characterized by monthly variations — a rise of temperature before menstruation and a fall during and after menstruation, with insignificant diurnal variations in the intermenstrual period.

On this account it is natural to expect that during pregnancy the processes of thermoregulation will be different from those at other times. Little work has been done however, on thermoregulation during pregnancy, and in the majority of cases this is concerned with mere statements that the temperature is raised during the first months of pregnancy [1, 3, 6, 9-11, 13 and others]. In certain investigations a fall in temperature before labor is mentioned [2, 4 and other].

The aim of the present research was to study experimentally the special features of the thermoregulation of the pregnant animal during general hypothermia and after administration of aminazine.

EXPERIMENTAL METHOD

Experiments were carried out on 128 pregnant and 63 nonpregnant white rats. Three series of experiments altogether were performed: in the first series of experiments the effect of aminazine alone was studied, in the second series — of a fall in the environmental temperature alone; in the third series of experiments the animals were cooled by a combination of administration of aminazine and the action of a low temperature of the surrounding air. The animals were cooled for 4-6 hours in a double-walled tank containing a cooling mixture (ice and salt) between the walls. The temperature inside the tank at the beginning of cooling was — 16° or — 12°. Aminazine was given in two doses: a minimum to show a hypothermic action, according to M. D. Mashkovskii, S. S. Liberman and A. I. Polezhaeva [5] — 0.55 mg per 100 g body weight, in the form of a 0.25% solution — and a larger dose — 1.2 mg per 100 g body weight in the form of a 0.8% solution.

In the experiments in which administration of aminazine was combined with cooling, the animals were kept in the tank immediately after receiving the drug. The temperature was measured by means of a thermocouple every 30 minutes. The animals were cooled and given aminazine at different periods of pregnancy: on the 4th, 12th and 21st days and the day before labor. In the analysis of the results the statistical significance of the findings was determined.

EXPERIMENTAL RESULTS

Administration of aminazine. This series consisted of 77 experiments. There was no great difference between the behavior of the pregnant and nonpregnant rats after receiving aminazine. Immediately after the injection some animals showed transient motor excitation, evidently the result of a reaction to the painful stimulus. From 30-60 minutes after the injection of aminazine they fell asleep and lay motionless. After administration of the larger doses of aminazine to the pregnant animals, they fell asleep after 15-30 minutes, whereas the nonpregnant animals fell asleep at the same time as after the smaller doses – after 30-60 minutes. The mean values of the changes in the rectal temperature as a result of injection of aminazine are shown in Table 1.

TABLE 1

Rectal Temperature of Pregnant and Nonpregnat Rats after Administration of Aminazine

Group of animals	Number of experiments	Initial rectal temperature	Fall in the rectal temperature in the 1st hour after injection of aminazine	Total fall in rectal temperature	Time of recovery, in hours
1. Aminazine in a dose of 0.55 mg per 100 g body weight					
Nonpregnant . .	18	36,64°	1,98° ±0,18°	3,47°	8,77
Pregnant:					
4th day	9	37,04°	2,13° ±0,3°	3,12°	6,39
12th "	15	36,71°	1,69° ±0,28°	4,28°	5,53
21st "	14	36,16°	0,93° ±0,12°	2,54°	7,08
2. Aminazine in a dose of 1.2 mg per 100 g body weight					
Nonpregnant . .	11	36,4°	2,96° ±0,17°	4,5°	—
Pregnant:					
21st day	10	36,13°	2,21° ±0,32°	5,73°	—

As may be seen from Table 1, the initial rectal temperature of the pregnant animals differed in accordance with the period of pregnancy: at the onset of pregnancy it was higher, and at the end – lower than in the nonpregnant rats. The greatest differences between the reactions of the pregnant and nonpregnant animals were shown on the 21st day of pregnancy, and were apparent within only one hour of the injection: in the nonpregnant animals the rectal temperature fell during the first hour by 1.98 ± 0.18°, and in the pregnant animals – by only 0.93 ± 0.12° (Fig. 1).

After administration of larger doses of aminazine the rectal temperature fell further in the pregnant rats on the eve of labor than in the nonpregnant rats.

Action of a low environmental temperature. In this series we carried out 39 experiments. During physical cooling the reaction of the pregnant animals was different from that found in response to injection of aminazine (Table 2).

The fall in the rectal temperature in the pregnant animals during the period of cooling was greater over the same length of time than in the nonpregnant rats (Figs. 1 and 2): In the nonpregnant rats during cooling for 4-6 hours the rectal temperature fell on the average by 2,2°, and in the pregnant rats during the same time it fell by 5.8-9.8° . The greatest fall of temperature was observed in the rats on the 21st day of pregnancy.

The difference between the falls in rectal temperature in the pregnant and nonpregnant animals was clearly apparent after only one hour of cooling, just as after injection of aminazine. For instance, in the rats on the 21st day of pregnancy the rectal temperature fell in the first hour of cooling on the average by 2,75 ± 0,65° but in the nonpregnant rats by only 0,82 ± 0,18° .

Restoration of the normal rectal temperature in the pregnant animals after cooling took place later than in the nonpregnant animals (see Table 2 and Fig. 2). Whereas in the nonpregnant animals a direct relationship was always observed between the duration of restoration of the rectal temperature and the degree of its fall, in the pregnant animals the duration of its restoration was independent of the degree of fall of the rectal temperature.

Combination of the action of aminazine and low environmental temperature. This series consisted of 75 experiments. Cooling was carried out until the rectal temperature was 20-24°. Immediately after being placed in the tank, both pregnant and nonpregnant rats showed some degree of motor restlessness, although in the majority of cases they rapidly quietened down and sat peacefully.

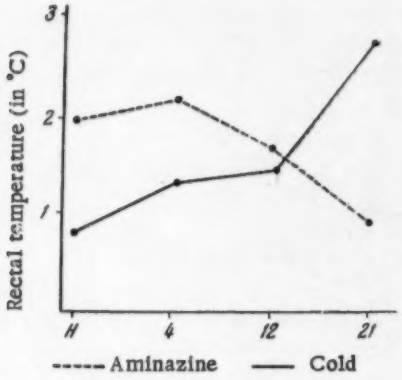

Fig. 1. Comparison of the degree of lowering of the temperature in pregnant rats under the influence of cooling for one hour and of aminazine (0.55 mg per 100 g body weight).
N — nonpregnant animals; 4, 12, 21 — pregnant animals, cooled on the 4th, 12th and 21st days of pregnancy respectively.

The results of the experiments are shown in Table 3.

The initial rectal temperature varied at different periods of pregnancy just as it did in the previous series of experiments.

With a combination of small doses of aminazine and a low environmental temperature, the differences observed between the reactions of the pregnant and nonpregnant rats to the isolated action of cold or aminazine were obliterated. The fall in the rectal temperature of these animals took place in the same way. Restoration of the temperature took slightly longer in the pregnant animals than in the nonpregnant (see Fig. 2).

The total fall in the rectal temperature of the rats in this series of experiments was greater than in response to the action of a low temperature or of aminazine alone (see Fig. 2). With a combination of larger doses of aminazine and a low environmental temperature, the temperature of the pregnant rats on the 21st day fell more in the 1st hour after the injection than in animals at the same period of pregnancy but receiving smaller doses of aminazine.

TABLE 2

The Rectal Temperature in Pregnant and Nonpregnant Rats during the Action of a Low Environmental Temperature

Group of animals	Number of experiments	Initial rectal temperature	Fall in the rectal temperature during 1 hour of cooling	Total fall in rectal temperature	Time of recovery, in hours
Nonpregnant	9	36.6°	0.82° ± 0.18°	2.23°	4.85
Pregnant:					
4th day	9	37.24°	1.32° ± 0.13°	6.63°	5.55
12th day	9	36.94°	1.49° ± 0.16°	5.82°	6.5
21st day	12	36.15°	2.75° ± 0.65°	9.82°	8.11

In the nonpregnant rats there was no difference between the fall of temperatures during the 1st hour after the injection of smaller and larger doses of aminazine (See Table 3).

Pregnant rats were thus more sensitive to larger doses of aminazine, both when given alone and when combined with cold.

The results of the experiments showed that the sensitivity of pregnant rats to a fall in the environmental temperature and to the administration of aminazine changed in opposite directions (see Fig. 1): The reactions to cooling were intensified and those to injection of aminazine were reduced. These relationships no longer held

in the case of extremely large doses of aminazine (1.2 mg per 100 g body weight), the administration of which to pregnant rats produced an even greater fall in the temperature than in the nonpregnant rats.

TABLE 3

The Rectal Temperature in Pregnant and Nonpregnant Rats during the Combined Action of Aminazine and a Low Environmental Temperature

Group of animals	Number of experiments	Initial rectal temperature	Fall in the rectal temperature in the 1st hour of cooling	Total fall in rectal temperature	Time of recovery, in hours
1. Combination of cold with aminazine in a dose of 0.55 mg per 100 g body weight					
Nonpregnat	15	36.38°	5.06 ± 0.65°	13.08°	11.93 ± 0.8
Pregnant:					
4th day	10	37.04°	4.03 ± 0.39°	14.72°	21.55 ± 4.48
12th day	15	36.7°	3.56 ± 0.33°	14.74°	21.9 ± 3.57
21st day	15	36.08°	5.41 ± 0.52°	13.02°	17.26 ± 2.28
2. Combination of cold with aminazine in a dose of 1.2 mg per 100 g body weight					
Nonpregnat	10	36.16°	5.28 ± 0.81°	10.76°	–
Pregnant:					
21st day	10	35.85°	7.71 ± 1.1°	13.04°	–

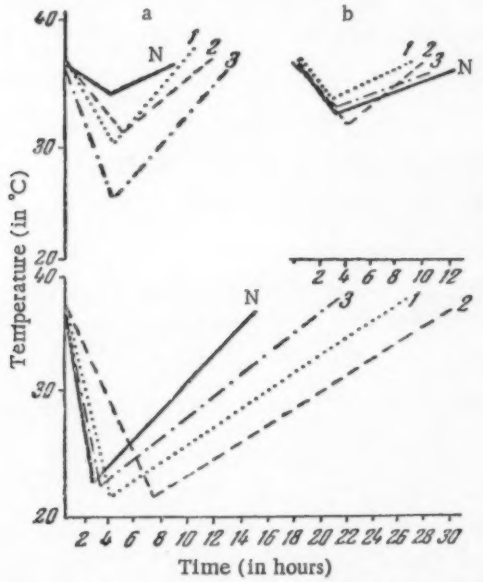

Fig. 2. Changes in the temperature of pregnant and nonpregnant rats in response to different methods of cooling.
a) cooling due to the action of a low temperature of the surrounding air; b) cooling due to administration of aminazine (0.55 mg per 100 g body weight); N) nonpregnant animals; 1, 2, 3) pregnant animals cooled on the 4th, 12th and 21st days of the pregnancy respectively.

Sensitivity to cooling could evidently be connected with the increasing development of a number of functions of the animal concerned in expulsion of the fetuses, the total weight of which often amounts to $\frac{1}{4}$ - $\frac{1}{3}$ of the weight of the rat itself; under these circumstances the weakening of its powers of adaptation to severe changes in the external conditions was understandable. So far as the distinctive features of the reaction of the pregnant animal to aminazine are concerned, it might be suggested that in association with the metabolic changes of pregnancy, because of the increased excitation of the corresponding centers of the brain aminazine does not have the same inhibitory action on these centers as it does in nonpregnant animals.

We know that during pregnancy, especially towards its end, considerable changes in metabolism are observed. According to A. A. Skvortsova, for instance, the basal metabolism and the gas exchange in pregnant women towards term is increased by $\frac{1}{3}$ by comparison with the metabolism of nonpregnant women [7]; a considerable increase in the respiratory gas exchange during the second half of pregnancy, notably at its end, has been pointed out by Zuntz [15], Klaften [12] and others; an increase in the basal metabolism during normal pregnancy has also been observed by Baer [8], Root and Root [14] and others.

SUMMARY

The peculiarities of thermoregulation were studied at various periods of pregnancy (on the 4th, 12th and 21st days) in administration of aminazine (chlorpromazine) and the action of low environmental temperature. The results of experiments demonstrated that the sensitivity of rats to low temperature and to the administration of aminazine (0.55 mg per 100 gms of body weight) is changed in the opposit direction at the end of pregnancy, i. e. it is increased under the effect of low temperature and decreased as a result of administration of aminazine. In simultaneous action of aminazine and low environmental temperature there is almost no difference in the effect produced by these agents on the gravid and nongravid animals.

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THE EFFECT OF OXYGEN DEFICIENCY ON THE DEVELOPMENT EXPERIMENTAL ATHEROSCLEROSIS OF THE CORONARY ARTERIES

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The role of oxygen deficiency in the development of atherosclerosis and cardiosclerosis has received inadequate attention from the point of view of experimental research.

In experiments on rabbits, R. L. Al'tshul' found that administration of cholesterol to animals kept in chambers with an increased oxygen content (up to 60-65%) greatly inhibits the development of atherosclerosis. In this author's opinion an increased supply of oxygen stimulates oxidation of cholesterol, and the oxycholesterol is either excreted from the body more readily or, passing with the bile into the intestine, does not undergo reabsorption as in the case of cholesterol.

The aim of the present research was to study the effect of prolonged oxygen deficiency on the degree and character of the atherosclerotic changes occurring in the coronary vessels during experimental cholesterol atherosclerosis.

EXPERIMENTAL METHOD

Experiments were carried out on 27 rabbits of the same sex, weighing 2000-2400 g, and kept under identical conditions on a standard diet.

The animals were subdivided into three groups.

The first group (10 rabbits) acted as controls, and received cholesterol (300 mg/ kg body weight) dissolved in vegetable oil, daily for 6 months.

The second group, like the first, received cholesterol in the same daily dosage, but at the end of the second month, in addition to feeding on cholesterol, the animals were kept each day for 3-6 hours in a chamber with a lowered oxygen concentration. The oxygen content of the chamber, whose volume was 250 cm^3, was brought down to 12% (by addition of nitrogen to the outside air). Air and nitrogen entered the chamber simultaneously and were mixed by an electric fan. Entry of the air and nitrogen was controlled by means of a kerosene rheometer. The concentration of oxygen and carbon dioxide in the chamber was determined by means of a Haldane apparatus.

The third group (5 rabbits) received no cholesterol, but at the end of the second month, like the rabbits of the second group, they were placed for 3-6 hours every day for 4 months in the chamber with the lowered oxygen concentration.

Every 15 days, before the rabbits were placed in the chamber and immediately after exposure to the low oxygen concentration for 3-6 hours, the blood cholesterol of all the animals was estimated by Grigaud's method.

TABLE 1

The Blood Cholesterol Concentration of the Rabbits of the First Control Group Receiving Cholesterol Alone

Rabbit No.	Weight		Blood cholesterol concentration (in mg %)												Degree of atherosclerosis	
				day of the experiment												
	before experiment	at the end of the experiment	before experiment	15th	30th	45th	60th	75th	90th	105th	120th	135th	150th	165th	180th	of the aorta	of the coronary arteries
95	2300	3100	84	260	326	482	642	814	940	1070	846	900	812	700	770	+++	++
96	2200	3500	76	350	474	530	620	780	830	930	760	822	750	680	610	+++	+
97	2300	2500	58	280	370	522	730	880	1060	1140	840	912	890	710	780	+++	++
98	2100	2800	42	212	286	336	400	450	512	420	450	516	536	580	470	+++	++
99	2200	2600	56	228	320	420	500	580	476	550	382	412	322	360	330	++	+
102	2100	3200	92	262	322	510	742	810	780	800	770	518	600	650	562	+++	++
103	2300	3500	68	312	440	622	864	928	1180	1280	1160	1050	870	840	810	+++	++
104	2000	3300	72	346	412	540	650	740	850	920	876	720	670	616	576	+++	++
105	2200	2800	88	210	356	410	542	710	890	850	740	580	550	630	680	+++	++
106	2000	3000	64	180	260	300	386	448	542	456	400	376	340	320	308	++	+
Average . . .			70	264	357,6	466,2	607,6	714	806	841,6	721,8	670,6	630	608,6	549,6		

TABLE 2

The Blood Cholesterol Concentration of the Rabbits of the Second Group, Receiving Cholesterol and Kept in a Chamber with a Low Oxygen Concentration

Rabbit No.	Weight		Blood cholesterol concentration (in mg %)												Degree of atherosclerosis	
	before experiment	at the end of the experiment	before experiment	day of the experiment											of the aorta	of the coronary arteries
				15th	30th	45th	60th	75th	90th	105th	120th	135th	150th	165th	180th	
68	2200	3100	76	328	600	772	856	1110	1420	1560	1680	1756	1610	1580	1558	++++	+++
69	2000	3200	94	344	428	660	780	1150	1530	1618	1534	1588	1670	1490	1350	++++	+++
78	2100	2900	82	270	362	448	534	700	946	1150	1390	1350	1440	1220	1146	++++	+++
79	2200	3250	68	242	326	400	580	770	884	970	854	920	784	850	986	++++	+++
84	2200	3300	76	226	412	430	380	562	684	642	560	620	462	570	462	++	+
86	2000	3500	58	300	390	380	458	760	810	872	990	960	916	924	844	+++	++
88	2300	3600	48	410	548	484	550	860	1160	1018	940	1084	1250	1176	1060	++++	+++
101	2200	3100	96	196	310	550	744	1026	1380	1260	1278	1210	1260	1320	1280	++++	+++
113	2100	3450	66	230	380	526	618	942	1000	1210	1324	1500	1540	1400	1312	++++	+++
118	2000	3200	74	310	486	622	826	1310	1450	1780	1600	1520	1470	1430	1500		
Average . . .			73,8	285,6	429,6	527,2	682,6	919,0	1126,4	1208	1215	1251	1240,2	1196	1149,8		

At the end of the sixth month all the animals were killed by air embolism and their aorta and heart were examined. The aorta was stained by Sudan III in toto, and serial sections of the heart were cut and stained by the usual methods.

The degree and character of the atherosclerosis of the aorta and coronary arteries of the heart were studied. The degree of atherosclerosis was designated conventionally by corsses: + alight changes; ++ moderate; +++ severe; ++++ most pronounced.

TABLE 3

The Blood Cholesterol Concentration of the Rabbits of the Third Group (Not Receiving Cholesterol), Kept in a Chamber with a Low Oxygen Concentration

Rabbit No.	Weight		Blood cholesterol concentration (in mg%)								
				day of the experiment							
	Before experiment	At the end of the experiment	Before experiment	15th	30th	45th	60th	75th	90th	105th	120th
183	2200	2900	82	98	74	102	96	86	88	100	96
184	2300	3000	76	88	96	90	94	78	96	92	88
186	2000	2800	54	78	92	86	98	94	88	96	76
187	2200	3000	92	104	96	110	94	108	102	94	100
201	2200	3200	66	84	92	102	86	100	98	100	94
Average			74	91	90	98	93	93	94	98	90

EXPERIMENTAL RESULTS

In the course of the experiment, for reasons unconnected with the experimental conditions, 2 rabbits of the second group died (in the 3rd and 4th months).

On the 15th day after the beginning of cholesterol feeding, the blood cholesterol level in the rabbits of the first, control group rose to 3-5 times its initial value, to reach its maximum in the 3rd-4th month of feeding, after which it gradually fell until the end of the experiment (Table 1).

In the experimental rabbits of the second group, cholesterol feeding plus keeping the animals in the chamber with a low oxygen concentration led to a sharp increase in the blood cholesterol, in individual cases up to 1780 mg%, which was maintained at a high level throughout the whole experiment (Table 2).

Thus, as a result of anoxia, the rise in the cholesterol concentration in the rabbits of the second group was significantly higher than that in the rabbits of the first, control group (see Table 1).

In the rabbits of the third group, which received no cholesterol but which were kept in the chamber with the low oxygen concentration, the blood cholesterol level rose insignificantly (Table 3).

Prolonged anoxia thus led to an increase in the blood cholesterol concentration, which was evidently due to a reduction in the processes of assimilation and synthesis of cholesterol and of the power to excrete it from the body.

In the rabbits of the first, control group the atherosclerotic plaques were situated mainly in the origin and the arch of the aorta, in the form of continuous outgrowths, markedly raised above the surface of the aorta. In the lower segments of the aorta isolated plaques were present, and where these became confluent, they formed separate areas of lipoidosis. The degree of atherosclerosis in the rabbits of the first, control group was assessed in the majority of cases as +++ (see Table 1).

In the rabbits of the second group atherosclerotic plaques were situated throughout the whole length of the aorta, including its abdominal portion. Merging with each other, they formed continuous zones of atherosclerosis, markedly elevated above the surface of the aorta in the form of outgrowths. The degree of atherosclerosis was assessed in the majority of cases as ++++ (see Table 2).

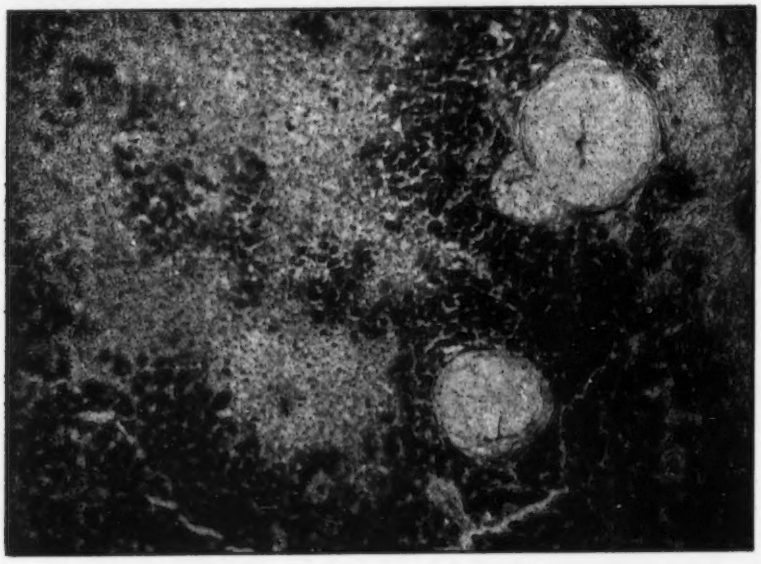

Fig. 1. Large atherosclerotic plaques in the intramuscular branches of the left descending coronary artery. Extensive area of severe destructive changes in the fibers of the myocardium.
Stained by Mallory's method. Objective 6×, ocular 7×. Rabbit No.69, Group — fed on cholesterol and kept in a chamber with a low oxygen concentration (12%). Duration of experiment 6 months.

In the third group no atherosclerotic changes could be found either macroscopically or microscopically.

Examination of the coronary arteries of the rabbits of the first, control group showed in the majority of cases a moderate atherosclerosis (++). Atherosclerotic plaques were situated mainly in the intramuscular branches of the left coronary artery. These atherosclerotic plaques greatly narrowed the lumen of the vessel, in some cases almost completely closing it.

Sometimes the atherosclerotic process mainly affected the subepicardial branches of the left coronary artery, but here they were mainly infiltrative in character; large plaques projecting into the lumen of the vessel were not found. From time to time the branches of the right coronary artery were affected also.

In the second group of rabbits the atherosclerosis of the coronary arteries was severe in the majority of cases (+++, see Table 2). Atherosclerotic plaques were found both in the intramuscular branches of the left coronary artery and in the main subepicardial trunks, projecting considerably into the lumen of the vessel.

In contrast to the control group, in the rabbits of the second group atherosclerosis affected almost all the subepicardial trunks of the left and, to some extent of the right coronary artery too. In the branches of the right coronary artery in the rabbits of the second group atherosclerotic plaques could be found, grossly narrowing the lumen of the vessel. The plaques consisted of large quantities of lipoid deposits, homogeneous or in places granular in structure. In the plaques there were strongly developed elastic fibers, and in places the internal elastic membrane was absent.

In the rabbits of the third group no atherosclerosis was present.

From our findings we concluded that oxygen deficiency in conjunction with cholesterol feeding caused a more pronounced hypercholesteremia and a severer degree of atherosclerosis of the aorta and the coronary arteries of the heart than feeding with cholesterol alone.

Oxygen deficiency evidently causes a disturbance of oxidative processes in the body, which in turn is reflected in the assimilation and synthesis of exogenous cholesterol. Without cholesterol feeding, oxygen deficiency raised the blood cholesterol concentration only insignificantly, and did not cause the development of atherosclerosis.

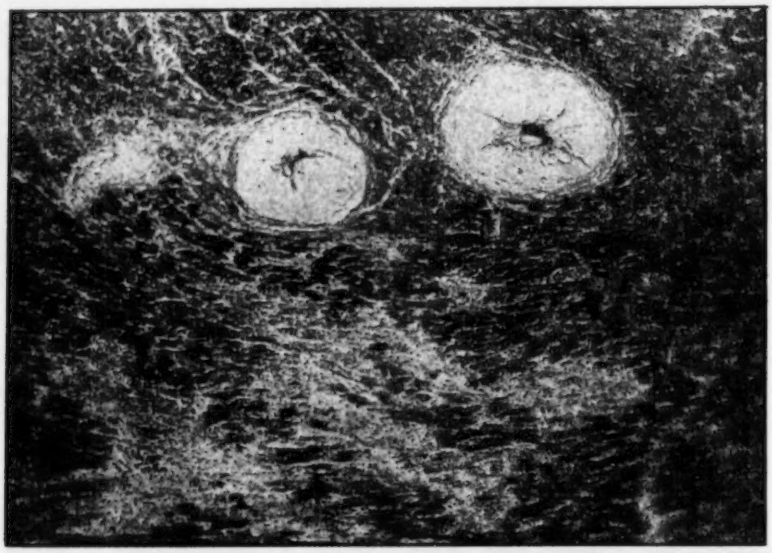

Fig. 2. Atherosclerotic plaques causing gross narrowing of the lumen of the intramuscular branches of the left coronary artery, near which can be seen well marked fibrotic lesions in the muscle of the left ventricle.
Stained by Mallory's stain. Objective 6×, ocular 7×. Rabbit No. 79, the same as in Fig. 1.

So far as myocardial changes were concerned, in the first, control group the atherosclerosis of the coronary arteries led in the majority of cases to moderate cardiosclerosis. In the heart muscle were observed small connective tissue scars, in places firm, in others friable, and mainly situated close to intramuscular arteries affected by atherosclerosis, with gross narrowing of the lumen. Areas in which fibers of connective tissue had developed were most often found in the anterior and anterolateral walls of the left ventricle.

In the second group, on examination of the heart muscle, extensive focal collections of cells with large number of lipoid inclusions in their protoplasm were discovered in the interstitial tissue of the mycoardium. These collections of cells were most often found in the papillary muscles. In places in the muscle of the left ventricle there was a diffuse lipoid infiltration of whole groups of muscle fibers.

Close to the arteries with atherosclerotic plaques and in other areas of the myocardium were observed gross lesions of a dystrophic or fibrotic character.

The dystrophic lesions consisted of destruction and necrosis of whole areas of muscle fibers. Many muscle fibers were severely vacuolated. The fibrotic lesions in the heart muscle of the rabbits consisted of multiple and extensive reticular scars.

Alongside areas of loose connective tissue were observed extensive, dense connective tissue scars, composed of coarse collagen fibers, staining intensively with picrofuchsin a crimson color. Some muscle fibers were separated by a large quantity of interstitial myocardial tissue, containing many cells. Among the areas of cellular infiltration were seen isolated cells undergoing destruction. The blood vessels were well filled and many capillaries and veins were dilated. The muscle of the ventricles, especially of the right ventricle, was hypertrophied.

The changes observed in the heart muscle of the rabbits of the second group could be regarded as a manifestation of diffuse cardiosclerosis with the formation of extensive areas of necrosis of whole groups of muscle fibers, as in the case of an infarct of the myocardium (Fig. 1). Here could be traced all stages of development of

sclerotic changes in the heart muscle, from areas of recent hemorrhage, destruction and lysis of muscle fibers up to scar tissue formation (Fig. 2).

Evidently in consequence of gross stenosing atherosclerosis of the coronary arteries and the action of anoxia, a severe disturbance of the normal blood supply of the heart muscle took place, with the development of extensive areas of necrosis. The diffuse atherosclerotic cardiosclerosis was severe (+++) in the rabbits of the second group.

In the third group of rabbits slight dilatation of individual capillaries and veins was observed in the heart muscle. In some areas of the left ventricle, close to its cavity, and in the papillary muscles solitary muscles fibers were found in a state of destruction. Small areas of proliferation of histiocytes were seen there also. No other abnormalities were found.

SUMMARY

Experiments were performed on rabbits. The author studied the effect of prolonged hypoxia on the development of aortic and coronary atherosclerosis in experimentally induced cholesterol atherosclerosis. Oxygen deficiency was attained by placing the animals daily into a chamber with decreased oxygen content (down to 12%) for 3-6 hours, for 4 months.

It was shown that prolonged hypoxia brings about a high hypercholesteremia and greatly intensifies the development of aortic and coronary atherosclerosis.

Prolonged hypoxia considerable increases stenosing atherosclerosis of the coronary arteries and causes severe dystrophic changes in the cardiac muscle in the form of numerous necroses of certain groups of muscular fibers and also always results in the development of diffuse cardiosclerosis in all cases.

THE EFFECT OF CHOLINE ON THE CHOLESTEROL METABOLISM OF STARVING RABBITS

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As shown by N. N. Anichkov and his co-workers [1, 2], the development of atherosclerosis is based on disturbance of the cholesterol metabolism. For this reason it must be regarded as an important task to discover substances which could restore to normal a disturbed cholesterol metabolism. In this respect great importance is attached to lipotropic factors and, in particular, to choline. However the reports in the literature on the role of choline in cholesterol metabolism are conflicting.

Morrison and his co-workers [15], Yu. T. Pushkar' [5] and T. A. Sinitsyna [6] pointed out that the administration of choline has a restraining action on the development of experimental atherosclerosis in rabbits fed on cholesterol. Morrison and Rossi [14] observed absorption of atheromatous plaques in rabbits as a result of the action of choline. T. A. Sinitsyna [7] showed the accelerating effect of choline on the regression of atherosclerotic lesions. Other workers, however, deny the importance of choline in preventing the development of experimental atherosclerosis in rabbits [8, 10], dogs [9] and fowls [16].

For this reason we carried out investigations in order to study the effect of choline on the development of hypercholesteremia in starving rabbits, and we also attempted to explain the mechanism of action of choline on this process.

The effect of choline on the development of hypercholesteremia in starving rabbits. For the study of disturbances of the cholesterol metabolism, a convenient experimental model is hypercholesterol in starving rabbits, since it develops rapidly and equally rapidly disappears when the rabbit is put back on a normal diet. The cholesterol level in the blood of starving rabbits varies considerably, and so in investigating the effect of choline on the development of hypercholesteremia in these conditions we carried out the experiments in such a way that the effect of choline could be compared in the same animal.

The experiments were performed on young rabbits of both sexes, weighing from 2200-2500 g. Blood was taken from the rabbit's heart, after which it was kept without food but was given water for 5 days. On the 3rd and 6th days of starvation blood was also taken and the cholesterol concentration in the plasma was estimated by Bloor's method and the lipoid phosphorus by the method of Fiske and Suffarow. After about 2 weeks, in the course of which the rabbit was kept on an ordinary diet, an analogous experiment was carried out again on the same animal, differing only in that the rabbit received choline daily in the form of a 10% solution of choline chloride in distilled water in a dose of 0.5 g per kg body weight (the choline was introduced into the stomach through a tube). In some animals the starvation experiments were carried out in the opposite order; the experiment giving choline was carried out first and was followed after the interval of time mentioned by an analogous experiment without choline.

In 18 of 25 experiments a well marked inhibition of hypercholesteremia was observed, in 4 experiments choline had no effect, and in three there was even a slight increase in the hypercholesteremia. The most characteristic results of these 18 experiments are shown in Table 1.

TABLE 1

Changes in the Blood Cholesterol Concentration in Rabbits on a Starvation Diet under the Influence of Choline

Experiment No.	Time of taking blood	Conditions of experiment					
		Starvation			Starvation + choline		
		Cholesterol (in mg%)	Lipoid phosphorus (in mg%)	Coefficient $\frac{1}{c}$	Cholesterol (in mg%)	Lipoid phosphorus (in mg%)	Coefficient $\frac{1}{c}$
1	Before starvation	20	3.5	4.3	23.5	4.0	4.1
	After 3 days of starvation	52.6	3.5	1.6	44.4	3.3	1.9
	After 4 days of starvation	71.1	3.8	1.3	50.0	4.1	2.0
2	Before starvation	44.4	4.0	2.2	40	3.9	2.4
	After 3 days of starvation	72.7	4.5	1.7	40	4.1	3.1
	After 5 days of starvation	100	4.0	1.0	46.6	4.1	2.2
3	Before starvation	40	3.5	2.1	40	4.1	2.5
	After 3 days of starvation	64	4.1	1.5	34.7	4.5	3.3
	After 5 days of starvation	88.8	5.0	1.4	36.7	3.8	2.6

Note: The coefficient $\frac{1}{c} - \frac{\text{lecithin}}{\text{cholesterol}}$

TABLE 2

The Effect of Choline on the Stability of the Blood Cholesterol of Rabbits Kept on a Starvation Diet

Experiment No.	Time of taking blood	Conditions of experiment									
		Starvation					Starvation + Choline				
		Before precipitation of proteins		After precipitation of proteins			Before precipitation of proteins		After precipitation of proteins		
		Cholesterol (in mg%)	lipoid phosphorus (in mg %)	Cholesterol (in mg%)	lipoid phosphorus (in mg %)	% of initial cholesterol concentration	Cholesterol (in mg%)	lipoid phosphorus (in mg %)	Cholesterol (in mg%)	lipoid phosphorus (in mg %)	% of initial cholesterol concentration
1	After 5 days of starvation	71.1	3.8	14.7	8.5	20.1	50.0	4.1	17.7	3.6	32
2	After 5 days of starvation	58.8	5	12.5	4.0	22	32	3.7	14.8	3.6	46.5
3	After 5 days of starvation	64	4.5	11.4	3.7	17.8	34.7	3.8	12.1	3.5	35.2
4	After 5 days of starvation	94.4	5.0	22.8	4.1	24.4	66.6	3.5	31.3	3.1	46.9
5	After 5 days of starvation	64	4.1	13.3	3.3	20.7	32	2.6	11	2.1	34.3

Table 3

The Effect of Choline on the Excretion of Cholesterol in the Bile

Experimental conditions	Number of experiments	Number of animals with a concentration of cholesterol in the liver of (in mg%)			
		38-50	51-100	101 and over	Average concentration
Starvation + choline	11	–	6	5	97.8
Starvation	9	4	3	2	68.1

TABLE 4

Results of Experiments to Study the Effect of Choline on the Cholesterol Metabolism in the Liver

Experimental conditions	Number of experiments	Number of animals with a concentration of cholesterol in the liver of (in mg%)				
		250-350	351-400	401-450	451 and above	Average concentration
Starvation + choline	19	4	3	6	6	416
Starvation	9	6	2	1	–	359

As may be seen from Table 1, the administration of choline to starving rabbits considerably retards the hypercholesteremia which develops under these circumstances. The concentration of lipoid phosphorus remained unchanged, although the coefficient l/c was considerably higher in the rabbits receiving choline than in those which were starved and did not receive choline, since the level of the cholesterol was lower in the experiments in which choline was given.

The effect of choline on the stability of the blood cholesterol in starving rabbits. There is no unanimity on the causes of the stability of the blood cholesterol level. Some authors [12, 13] account for the stability of the blood cholesterol by its combination with proteins, others [4, 11] by its combination with phosphatides. Since the coefficient l/c in all the experiments with starving rabbits which received choline was significantly higher than in the experiments on the starving rabbits not receiving choline, it appeared that this must be the feature which led to the greater stability of the blood cholesterol of these animals.

Experiments were carried out by a method described in a paper by S. V. Nedzvedskii and S. S. Ratnitskaya [4]. The results of some typical experiments are shown in Table 2.

The results in Table 2 show that the administration of choline to starving rabbits increased the stability of the blood cholesterol: after precipitation of the plasma proteins, 24-69% of the total cholesterol remained in the protein-free filtrate whereas in the experiments in which no choline was given, this figure was 15-27%.

The effect of choline on the excretion of cholesterol in the bile of starving rabbits. Many authors have pointed out that the liver plays an essential part in the excretion of cholesterol with the bile. We therefore considered it of interest to study the effect of choline on the cholesterol concentration in the bile.

Two groups of rabbits were used in these experiments. Animals were given choline daily (0.5 g per kg body weight), and after starvation for 5 days, were killed by air embolism; their bile was collected in a tube and the cholesterol concentration of the bile estimated accurately by the method of Sperry and Brand. Similar experiments were carried out on rabbits which received no choline during the period of starvation.

Table 5

The Effect of Choline on the Cholesterol Content of Liver Tissue Incubated under Various Conditions

Experiment No.	Experimental conditions	Cholesterol (in mg%)		
		Control	Experiment	
		Incubation of liver tissue		
		with NaOH, 15%	with CH_3COOH, 5%	with KH_2PO_4, 1%
1	Starvation + administration of choline	500	454	384
2	Starvation + administration of choline	395	416	326
3	Starvation + administration of choline	470	470	390
4	Starvation + administration of choline	400	363	333
5	Starvation + administration of choline	454	454	400
6	Starvation without choline	333.3	327	327
7	Starvation without choline	347.8	347	347
8	Starvation without choline	307	307	301
9	Starvation without choline	250	250	250
10	Starvation without choline	400	400	400

The results of these experiments are shown in Table 3.

It can be seen from Table 3 that the cholesterol content of the gall bladder was higher in the majority of the starving rabbits receiving choline than in the starving rabbits not receiving choline. Administration of choline therefore increased the cholesterol content of the gall bladder of starving rabbits, thereby facilitating it excretion.

The effect of choline on the cholesterol metabolism in the liver of starving rabbits. Recent work has shown the important role of the liver in cholesterol metabolism. Since choline reduces hypercholesteremia in starving rabbits, the question arose whether this phenomenon depended on the cholesterol metabolism in the liver of these rabbits. This appeared more likely since it had been established in a recently published paper by V. N. Kolmakov [3] that there was a relationship between the fall in the hypercholesteremia and the cholesterol metabolism in the liver of starving rabbits under the influence of vitamin C. We therefore studied the effect of choline on the cholesterol content of the liver in starving rabbits. These experiments were carried out as described in the paper cited [3]. In all 28 experiments were performed, in 19 of which choline was given to starving rabbits, and in 9 no choline was given (the number of the latter experiments was smaller because the results were the same as those obtained on this subject in earlier experiments in our laboratory). In Table 4 are shown the results of these experiments.

As can be seen from Table 4, the cholesterol content of the liver of starving rabbits which were given choline was higher in the majority of cases than in those which did not receive choline. It could therefore be considered that under the influence of choline the liver had a greater restraining action on the blood cholesterol, which led to a fall in the hypercholesteremia. The higher cholesterol content of the liver could possibly be due,

however, to the fact that in these conditions the synthesis of this substance is stimulated or its breakdown is inhibited in the liver. In order to clarify this point we carried out experiments in which the liver tissue of experimental animals was incubated: 1) with acetate, where synthesis of cholesterol was postulated, and 2) with potassium phosphate, where breakdown of cholesterol was postulated, as described by V. N. Kolmakov [3]. The results of some typical experiments are given in Table 5.

It follows from the results given in Table 5 that both the synthesis and breakdown of cholesterol are inhibited in the liver of starving rabbits. The administration of choline to starving rabbits restored the breakdown of cholesterol in the liver tissue but had no essential effect on its synthesis.

It may thus be considered that under the influence of choline the power of the liver to store and break down the cholesterol of the blood is increased, which leads to a reduction of the hypercholesteremia.

It may be concluded from these experiments that the administration of choline to starving animals delays the development of hypercholesteremia, increases the stability of the blood cholesterol of these animals, promotes storage by the liver of the cholesterol of the blood and the excretion of cholesterol in the bile, and stimulates the breakdown of cholesterol in the liver.

SUMMARY

The effect of choline on the cholesterol metabolism was studied in fasting rabbits. Administration of choline (0.5 gm per kg of body weight) delays the development of hypercholesteremia, increases the resistance of cholesterol in the plasma of these animals, promotes the retention of blood cholesterol by the liver and cholesterol excretion in the bile and intensifies the processes of cholesterol distruction in the liver.

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FRACTIONATION OF DESOXYRIBONUCLEOPROTEINS ISOLATED FROM THE LIVER

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Investigations during recent years have shown that desoxyribonucleic acid (DNA), isolated from a single biological source, consists of a group of closely connected polymers, differing essentially in their structure. Several authors [2, 4, 5, 6] have succeeded in separating DNA into several fractions with different proportions of nitrogenous bases. The methods of fractionation which have so far been used may be subdivided into the following groups:

1) extraction of denatured desoxyribonucleoprotein (DNP) with sodium chloride solution and paying attention to the concentration gradient [4, 6] or the time gradient [9];

2) fractionation of the isolated DNA by treatment with ion-exchange reagents [2], with polypeptides [12] or with proteins [3];

3) fractionation of denatured DNP by replacement of the protein by powerful anionic polymers – heparin and dextran sulfate [7];

4) fractionation by means of paper electrophoresis [11].

It is mainly the DNP from microorganisms that has been subjected to fractionation by the above methods. Less work has been done on the fractionation of DNP isolated from animal tissues; fractionation here of the DNP obtained from the liver by extraction with saline has not been undertaken at all.

The aim of the present work was to carry out fractionation of DNP obtained from the liver. For our attempt we utilized the simplest methods of saline fractionation with NaCl solution of constant or varying concentration, in the cold and at different temperatures. We suggested this last modification of saline fractionation, using different temperatures, and worked out the details in this particular investigation in view of the idea that the stability of the bond between protein and nucleic acid varies with the temperature.

EXPERIMENTAL METHOD

We obtained the DNP from the ground-up liver of cattle and rabbits by the method of Mirsky and Pollister [10]. The liver was taken without delay after the animals had been slaughtered and was kept frozen at −20°. The product was precipitated 3 times from 6 volumes of distilled water until the N/P ratio was 3.6-3.7. The DNA content of the preparations was estimated as phosphorus (by the method of Fiske and Subbarow) and as nitrogen (by Conway's method).

The solution of DNP in 1 M NaCl was precipitated before fractionation in 6 volumes of distilled water. To the gel was added an equal volume of NaCl solution of concentration required by the conditions of the experiment,

and the preparation was kept in the refrigerator for 30 minutes, being stirred slowly all the time. After 30 minutes a mixture of chloroform and butyl alcohol (3:1) was added, in a volume equal to one half that of the gel solution, and the whole was mixed for 2 minutes in the cold by means of a high-speed mixer. The emulsion thus obtained was centrifuged in the cold at 3000 rpm for 10 minutes.

TABLE 1

Fractionation of DNP from Ox Liver by NaCl Solutions of Different Concentration at 5°

Material examined	Concentration of NaCl	Content of		N/P	a/t	g/c	$\frac{a+g}{t+c}$	$\frac{a+t}{g+c}$
		P per ml (in γ)	N per ml (in γ)					
Whole nucleoprotein	—	132	488.4	3.7	0.95	1.11	1.02	1.28
	1. Group of experiments							
1st fraction	0.5 M	54.6	220.8	4.04	0.98	1.11	1.03	1.31
2nd fraction	0.9 M	60.2	125.3	2.08	0.94	1.13	1.02	1.33
3rd fraction	1.3 M	13.0	47.7	3.66	1.03	1.07	1.05	1.24
	2. Group of experiments							
1st fraction	0.35 M	10.3	35.8	3.45	0.87	1.17	0.99	1.34
2nd fraction	0.5 M	138.0	256.0	1.85	0.96	1.15	1.05	1.05
3rd fraction	0.7 M	44.5	161.0	3.61	1.06	1.06	1.08	1.15
4th fraction	0.9 M	10.7	35.7	3.33	1.99	1.07	1.06	0.82

Symbols used in this and later tables: a — adenine, t — thymine, g — guanine, c — cytosine.

The supernatant fluid was the first fraction. The protein gel formed with part of the unfractionated nucleoprotein was treated by the same method, but when different temperatures were used the last 30-minute extraction took place at 20° (for the second fraction) and at 37° (for the third fraction). During fractionation with NaCl solution of constant concentration, the same 0.5 M solution was used for each successive fractionation; during fractionation with NaCl solutions of different concentration, this was successively increased. The concentration of DNA, protein, purines and pyrimidines in the fractions thus obtained was estimated by paper chromatography, using A. S. Spirin's [1] modification of Wyatt's method [13].

Each fractionation was usually repeated twice. In the chromatographic analysis, 2 parallel samples of hydrolyzate were taken from each fraction, and 2 chromatograms were taken of each sample (the mean values are shown in the tables).

EXPERIMENTAL RESULTS

In the course of our research we at first believed that fractionation would be most successful when using NaCl solutions of different concentration. In the first group of experiments, fractionation was therefore carried out by the use of NaCl solutions in concentrations of 0.5, 0.7 and 1.3 M, and in the second group — of 0.35, 0.7 and 0.9 M at 5°. The results of these experiments are shown in Table 1.

As may be seen from Table 1, in the first group of experiments 3 DNA fractions were obtained with an insignificant difference in the composition of their nitrogenous bases, and in the second group 4 fractions were obtained, of which the second and third differed only slightly.

No form of sequence was observed in the changes in the specificity factor of the nitrogenous bases ($\frac{a+t}{g+c}$).

Thus, from our point of view, this fractionation did not give satisfactory results. We therefore carried out experiments using solutions of constant concentration for the fractionation. Under these circumstances we obtained (see Table 2, 1st method) 3 fractions with an insignificant difference between the second and third fractions,

i. e. this method also, according to our view, fails to give sufficiently clear fractionation of the DNA. We thereupon carried out experiments in which fractionation of DNP from ox liver was done at different temperatures and with a constant concentration of NaCl. In this case clear differences were found between all three fractions, with a successive fall in the specificity factor from the first to the third (see Table 2, 2nd method).

TABLE 2

Fractionation of DNP from Ox Liver with 0.5 M NaCl Solution at Constant and Differing Temperatures

Material examined	Temperature	Content of P per ml (in γ)	Content of N per ml (in γ)	N/P	a/t	g/c	$\frac{a+g}{t+c}$	$\frac{a+t}{g+c}$
Whole nucleoprotein	—	168.0	605.0	3.6	0.97	1.08	1.02	1.25
		1st method						
1st fraction	5°	23.6	98.9	4.20	1.07	0.94	1.02	1.32
2nd fraction	5°	37.1	98.9	2.66	1.02	1.09	1.05	0.99
3rd fraction	5°	12.0	59.6	4.96	1.04	1.08	1.06	1.06
		2nd method						
1st fraction	5°	23.5	86.1	3.66	0.95	1.05	1.00	1.35
2nd fraction	20°	29.2	92.7	3.17	0.93	1.10	1.00	1.25
3rd fraction	37°	12.0	66.2	5.52	0.95	1.06	1.03	0.74

TABLE 3

Fractionation of DNP from Rabbit Liver with 0.5 M NaCl Solution at Different Temperatures

Material examined	Content of P per ml (in γ)	Content of N per ml (in γ)	N/P	a/t	g/c	$\frac{a+g}{t+c}$	$\frac{a+t}{g+c}$	Temperature
Whole nucleoprotein	86.0	320.0	3.72	0.98	1.08	1.02	1.19	—
1st fraction	41.5	140.0	3.37	0.96	1.03	0.99	1.20	5°
2nd fraction	13.3	35.0	2.63	1.04	1.05	1.05	0.99	20°
3rd fraction	20.8	70.0	3.36	1.10	1.08	1.09	0.85	37°

In order to test the reproducibility of this method for the liver DNP of another species of animal, we carried out fractionation experiments on DNP isolated from the liver of the rabbit. Table 3 shows that in this case too, the 3 fractions obtained had a different specificity factor, its value falling progressively from the first to the third fraction.

From a comparison of the methods of fractionation with 0.5 M NaCl at constant and different temperatures (see Table 2), it can be seen that the first fraction from the first method and the first fraction from the second method, obtained under practically the same conditions and at the same temperature, resembled each other very closely in their specificity factor, as was to be expected. Subsequent changes in the temperature, however, led to the production of fractions quite different in their composition from those obtained by the first method. This suggested that the method of fractionation which we were using was effective.

A control determination of the viscosity showed the high degree of polymerization of the test preparations and their fractions. The ratio of purines to pyrimidines in all the fractions was close to unity, i. e. was in agreement with Chargaff's rule with all the methods of fractionation.

When the results were compared with those reported in the literature, some lack of correspondence was observed with the results of saline fractionation described by a number of workers [4-6], for in their research an increase was observed in the coefficient of specificity from the first fractions to the last. Our method of fractiona-

tion differed, however, from the methods used by the authors cited, chiefly by the nature of the initial preparation. We thought that for fractionation it was necessary to select nucleoprotein preparations of sufficient stability, for only these would have the characteristic composition of the particular tissue. Accordingly, by triple precipitation we freed the initial nucleoprotein from weakly combined and possibly contaminating fractions, and obtained in every case preparations which were soluble in 1 M NaCl and with N/P = 3.6-3.7, i. e. in the proportion characteristic of purified nucleoprotein. According to the literature the initial nucleoproteins usually selected for fractionation were water-soluble preparations with a higher N/P ratio.

It must also be pointed out that the preliminary dissolving of the initial preparation in 1 M NaCl in our own investigation could possibly increase the lability of the bonds between the protein and nucleic acid with an increased content of adenine and thymine. It is interesting to point out that, according to Kent [7], during the fractionation of nucleoprotein dissolved in 1 M NaCl with heparin and dextran sulfate, the same sequence was observed in the changes in the specificity factor through the fractions as in the present work.

We therefore have demonstrated the possibility of saline fractionation of the DNP obtained from the liver of cattle and rabbits; we have suggested a new method of fractionation of DNP at different temperatures; and we have obtained fractions of nucleic acid which differed clearly from each other in the factor of specificity of composition of nitrogenous bases, which decreased from the first fraction to the last.

SUMMARY

The possibility of the salt fractionation of desoxyribonucleoproteins (DNP) obtained from the liver of cattle and rabbits was demonstrated. A new method of fractionation of DNP at various temperatures was suggested. The authors obtained three fractions of nucleic acid which have a pronounced difference from each other by the specific content of the nitrous bases which decrease from the first fraction to the next.

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MITOGENETIC ANALYSIS OF THE PROTEIN FRAMEWORK OF PROTOPLASM COMMUNICATION III. SPECIAL FEATURES OF THE FRAMEWORK OF THE CANCER CELL

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In previous papers [4, 5] it has been shown that the protein framework of tissues in which cell division is proceeding is characterized by a reduction in the number of weak intermolecular bonds, i.e. an increase in the independence and, hence, of the mobility of the peptide chains over that found in tissues emerging from a meristematic state.

It seemed probable that the character of the protein framework of the cancer cell would be rather different. We know that the great difference between cancer tissue and normal meristems is shown, not by an increase in the mitotic coefficient, but the continuousness of division and, at the same time, by the topographical unrestraint of mitosis in the malignant foci, and the dissemination of mitoses throughout the tumor, in contrast to the confined nature of the foci of proliferation of normal meristems. It might have been expected that the protein framework of the cancer cell would also be distinguishable by some sign from the framework of the normal dividing cell. The present investigation is devoted to the partial explanation of this problem.

EXPERIMENTAL METHOD

We investigated implanted mouse adenocarcinoma (produced by subcutaneous inoculation of 0.2 cm^3 of a suspension of ascitic cells in the dorsal region), both in vivo and in isolation from the animal. The mitogenetic radiation of the tumor was studied on the 8-11th day after implantation.

For the experiment the mouse was fixed on a small operation table. A flap of skin covering the tumor was reflected, and the small exposed surface (approximately 3 × 3 mm) was centred in front of the collimator of the spectrograph (all the remaining surface of the tumor and adjacent areas of the trunk were covered). The tumor which had been isolated from the animal was treated in a similar way. Throughout the experiment the tumors were bathed in physiological saline, warmed to 38-40°.

As had previously been shown [1-3], tumors not separated from the body produce a constant and intensive (within the limits of mitogenetic intensity) spontaneous mitogenetic radiation. For this reason the method of spectral analysis of selective dispersion, as described in the previous communications [4, 5], could not be used in this part of the investigation.

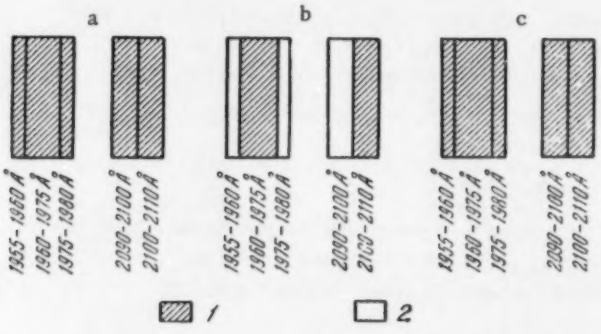
We studied the spectral bands corresponding to the emission from the carbonyl R – C – O and oxyphenyl R – C_6H_4 OH groups, and the bands of the peptide end groups R – OH and R – NH_2.

EXPERIMENTAL RESULTS

Even the first results showed a clear difference in the state of the protein frameworks of the malignant tumor and normal tissue.

The spectral bands for the carbonyl and oxyphenyl groups of the cancer tissue were wider than the standard values for these bands, and than the identical bands found during investigation of the radiation from normal tissues (see Figure).

In the normal tissues of the mucous membrane of the lip of a newborn guinea pig, the cells of which are capable of division, the R − C = O group was represented by bands between 1960-1975 A and between 2100 and 2110 A. In the tissues of the labial mucosa of the adult guinea pig, emerging from a meristematic state, these radicals also emit radiation in neighboring areas of the spectrum. The field of irradiation was thus widened. The field of irradiation of adult guinea pig tissue was 1955-1980 A and 2090-2110 A. The degree of widening of the bands which characterized the radiation emitted from cancer tissue was analogous to the widening of the same bands observed in the radiation emitted by tissues emerging from a meristematic state.



CO bands in the selective dispersion spectrum of adenocarcinoma and normal tissue.
a) Adenocarcinoma; b) mucous membrane of the lip of a newborn guinea pig; c) mucous membrane of the lip of an adult guinea pig; 1) active band; 2) inactive band.

From these findings the following direct conclusion could be drawn. A typical feature of the protein framework of the cancer cell was, evidently, the considerable preponderance of weak intermolecular bonds, joining the peptide chains in dimeride and trimeride systems, i.e. it was typified by a certain degree of aggregation of the framework, analogous to its condition in normal tissues in which no cell division was present.

This conclusion appeared to conflict with our views on the mobility of the protein and peptide systems of the framework of the dividing cells. The results of later spectral analysis of bands characterizing the peptide end groups $R-NH_2$ and R − OH went a long way towards removing this contradiction.

Investigation of the mitogenetic radiation of cancer tissue clearly revealed bands characteristic of amino- and hydroxyl groups at exposures of 21-24 seconds. This indicated that their amount was comparatively great, i.e. that the peptide chains undergoing aggregation were relatively short*. In normal tissue, containing dividing cells, from the labial mucosa of a young guinea pig, these bands were found at even lower exposures − 10-12 seconds. To judge by the intensity of the bands of malignant and normal tissue, the results can be arranged in the following order (see Table).

In the normal tissue of the adult animal we found no bands characteristic of amino- and hydroxyl groups at exposures of 6-40 seconds. The absence of bands at these exposures demonstrated that the intensity of radiation from the labial mucosa of the adult guinea pig was significantly less than that from normal tissue containing dividing cells and from cancer tissue.

* The criteria of the intensity (clarity) of the bands was the threshold of effective exposure. It was found experimentally that the intensity of the radiation and the threshold exposure were inversely proportional (It = constant).

In cancer tissue these groups were found at exposures of 21-24 seconds, and in tissue from the lip of a newborn guinea pig, at exposures of 10-12 seconds. From comparison of the results, the following concept emerges: aggregation of the protein framework is expressed in the cancer cell in a perfectly definite form – a predominance of short peptide complexes. Under this term we imply the combination of short segments of the peptide chains in dimeride, or even trimeride aggregates. Such a state may correspond to the considerable mobility of these peptide complexes, although it is possible that by comparison with normal dividing cells, characterized by free, short peptide chains [5], their mobility is slightly reduced.

Characteristic Radiation of Peptide End Groups

	Test object							
	mucous membrane of the lip of an adult guinea pig		mucous membrane of the lip of a newborn guinea pig		mouse adenocarcinoma			
					with no other agent		after administration of an extinguisher	
	effect (in %)	exposure (in seconds)	effect (in %)	threshold exposure (in seconds)	effect (in %)	threshold exposure (in seconds)	effect (in %)	threshold exposure (in seconds)
R – NH_2:								
2065-2070 A	2	6-40	41	12*	76	24	57	32
The same	–1	6-40	42	10	42	21	37	32
The same	1	6-40	35	12	50	21	38	32
The same	4	6-40	45	12	41	21	42	32
The same	3	6-40	43	10	36	21	40	32
2260-2270 A	1	6-40	32	12	47	24	75	32
The same	–2	6-40	46	10	30	21	36	32
The same	1	6-40	38	12	53	21	32	32
The same	–9	6-40	36	12	47	21	49	32
The same	–2	6-40	45	10	42	21	35	32
The same	5	6-40	51	10	44	24	48	35
R – OH:								
24-2450 A	–	–	49	10	46	24	52	32
The same	–	–	34	12	44	24	47	32
The same	–	–	43	10	55	21	49	32
The same	–	–	–	–	59	21	–	–

* At lower exposures – no effect.

In addition, however, the idea is very likely that directly after the onset of mitosis in the cancer cell, disintegration of the short aggregates takes place, i.e. free, short peptide chains are formed, analogous to those which predominate in normal dividing cells. The mobility of the framework of the various groups of cells would then be strictly comparable.

Some grounds for this hypothesis are provided by the following simple consideration: During the study of the mitogenetic spectra of normal and malignant tissues, we recorded the radiation of a large aggregation of cells, the majority of which, of course, were in a state of interkinesis, and a smaller number in the phases of mitosis. In other words, the results obtained related mainly to cells in different stages of preparation for division; we have no direct grounds for extending this criterion to the stage of mitosis also. Whether we adopt this point of view or not, however, it is obvious that the state of interkinesis of normal tissues and cancer tissue is typified by considerable difference in the spatial relationships of the structure of the protein framework.

In connection with the results obtained, the following question arose: Was the characteristic state of the protein substrate of the cancer cell associated to some extent with a distinctive mitogenetic pattern?

Whereas normal meristems are characterized by premitotic emission of radiation only, a continuous mitogenetic emission is specific for cancer tissue, probably due mainly to the presence in the cell of a continuously radiating carcinogen (unpublished work from this laboratory). We are not yet able to supply a sufficiently well-grounded or complete answer to this question, but in accordance with other findings, obtained some time ago in this laboratory [1, 2], it seems very likely to us that the small size of the peptide chains in the cancer cell is connected with the continuity of the mitogenetic radiation.

It was found that peptide synthesis was stimulated by mitogenetic radiation both in vivo and in experiments with aminoacid solutions, and that under these conditions, i.e. during excitation of the protein framework with high quanta of energy, the formation of peptide chains acquired the character of a chain reaction. At the same time it was found that intermittent radiation had the greatest stimulating effect; a continuous flow of photons, on the other hand, leads to frequent rupture of the developing chains.

In order to test the hypothesis of the importance of radiation for the state of the protein framework of the cancer cell, the cancerous mouse was injected subcutaneously with a so-called extinguisher of mitogenetic radiation – a solution of furfurol, which absorbs almost the entire ultraviolet region of the spectrum; this was injected in very small concentration – of the order of 10^{-6}, in a dose of 0.5 ml.

A fall in the genera intensity of radiation, especially after several repeated injections, and a fall in particular in the radiation from the malignant tumor, led to the expected result (see Table, last column). With the aid of spectral mitogenetic analysis it was found that after injection of the extinguisher a considerable weakening was observed of the characteristic bands of the peptide end groups (NH_2), whereas, for example, the bands of the carbonyl group did not alter in intensity. In these experiments we used the method of selective dispersion of mitogenetic radiation, which is known to allow the spectra of the functional groups to be detected even in an unirradiated or a feeble irradiated protein framework. When the mitogenetic emission is weakened, the length of the peptide chains in the cancer cells isthus increased.

In other words, the above-mentioned assumption that shortening of the chains was due to continuous radiation, specific for the cancer cell, was placed on a firm footing. We may add that a specific feature of the protein framework of the cancer cell is the predominance of dimeride and even trimeride systems of peptide chains, joined by intermolecular bonds, but of no great length and, hence, mobile. The mobility of the protein framework is one of the essential conditions of mitosis.

A fall in the continuous mitogenetic emission from the cancer cell, which may be brought about by injecting the animal with an extinguisher of mitogenetic radiation, leads to a significant lengthening of the chains, thereby to some extent bringing the state of its protein framework nearer to that of normal cells, not in process of division.

SUMMARY

By the method of mitogenetic spectral analysis it has been demonstrted that the protein substrate of cancer cells differs from the substrate of the normal tissue in which cellular division occurs. In 21-24 second exposures amino- and hydroxyl end groups have been revealed in the cancer tissue. Irradiations characterizing these radicals are found in 10 – 12-second exposures of the tissues of newborn guinea pigs containing dividing cells. At the same time the mitogenetic spectri of the cancer tissue characterizing the carboxyl (R – C – O) and the oxyphenyl (R – C_6 – H_4OH) groups are widened in comparison with the bands peculiar to the normal tissues with dividing cells. Hence it may be assumed that the cancer cell substrate consists of short (with relation to the main axis) two and "three-dimentional agreggates". Administration of the mitogenetic radiation extinguisher (fufurol solution) to mice has demonstrated the short chains to be related to the constancy of the radiation specific for cancer cells.

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THE DISTRIBUTION OF ALKALINE AND ACID PHOSPHATASE IN THE LIVER OF WHITE RATS DURING IRRADIATION WITH THE RADIOACTIVE ISOTOPE OF IRON Fe^{59}

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There are already several reports in the literature on the influence of x-ray irradiation on the alkaline and acid phosphatase activity of different organs (see the monograph by M. N. Pobedinskii [1]).

M. A. Presnov (see [1]), for instance, studied the alkaline and acid glycerophosphatases of tissues of white rats by a histochemical method, during irradiation with x-rays, and came to the conclusion that general irradiation of the animal leads to increased alkaline and decreased acid phosphatase activity in certain tissues.

Histochemical investigations of the change in the alkaline phosphatase activity of the liver during irradiation of the rabbit's skull with x-rays were carried out by Cali and Verga [3]. These authors found an increase in the glycerophosphatase activity of the liver 24 hours after irradiation. Lelièvre, Betz, et al. [5], studying the changes in the liver of mice after total irradiation with x-rays in large doses, came to the conclusion that the distribution and concentration of acid and alkaline phosphatase in the liver of the irradiated animals showed no change from those of the controls.

Mention must be made of the work of Z. S. Tepikina (1957) [2], who studied by a biochemical method the effect of Fe^{59} on the metabolism in the liver after administration of the radioisotope to animals. The experiments of this author showed in response to internal irradiation, in addition to other changes in the liver, increased activity of the acid phosphatase.

In the accessible literature we were unable to find any histochemical investigations devoted to the study of the phosphatase activity of the organs and tissues in response to internal irradiation by the radioactive isotope Fe^{59}. Such information would, however, be of practical importance.

In the present research, we investigated the activity of the alkaline and acid phosphatases in the liver after internal administration of the radioactive isotope of iron Fe^{59} to animals.

EXPERIMENTAL METHOD

Experiments were carried out on white rats of both sexes, weighing 150-200 g. The experimental animals received 25-45 μC of the radioactive isotope of iron Fe^{59} each day with their food for a predetermined time. Experimental and control rats were killed 6 days after the administration of the isotope began. Altogether 39 rats were used, 21 experimental and 18 control. As soon as the animal had been killed the liver was fixed in 80° alcohol*, and the alkaline and acid phosphatases were determined in frozen and paraffin wax sections by Gomori's [4] method. Sections were incubated in substrates for 3 and 20 hours respectively. The sites of activity of alkaline phosphatase stained a black color, and the acid – brown.

* This part of the work was undertaken by Z. S. Tepikina of the Department of Biochemistry.

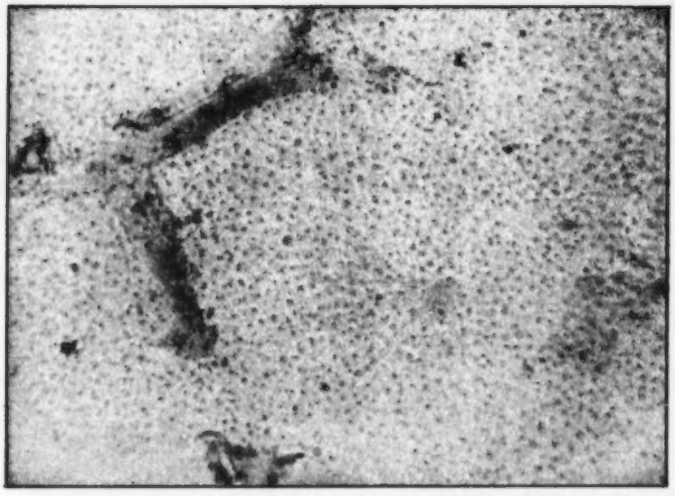

Fig. 1. Distribution of alkaline phosphatase in the liver of control rats. Treated by Gomori's method. Objective 8 ×, ocular 7 ×.

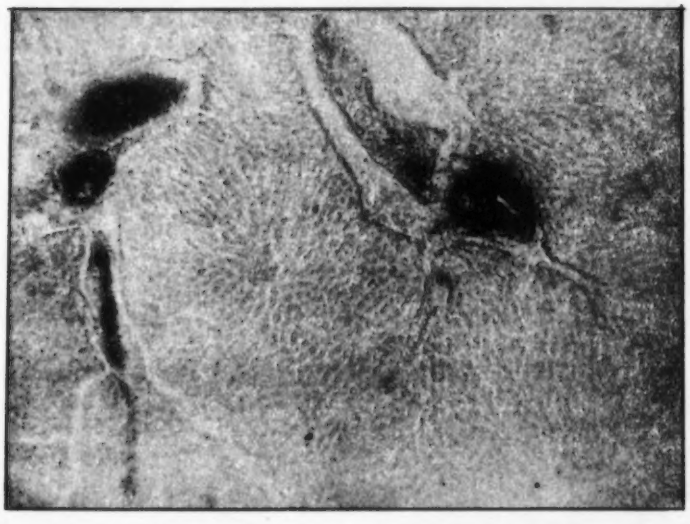

Fig. 2. Alkaline phosphatase in the wall of the interlobular bile ducts. Liver of an irradiated rat. Treated by Gomori's method. Objective 8 ×, ocular 7 ×.

EXPERIMENTAL RESULTS

In the liver cells of the control rats, the enzyme was found in the nuclei, mainly in the nucleoli, although cells were found which did not contain phosphatase. Such cells were distributed mainly around the central veins.

In the lumen of the blood vessels of the liver were found leucocytes with a very high concentration of alkaline phosphatase, and there were more of these cells in the septal veins (Fig. 1). Leucocytes were found in smaller numbers in the blood capillaries, and only solitary ones in the lumen of the central veins. It should be pointed out that the nuclei of the liver cells were more blackened in the areas in which were the largest collections of leucocytes.

High alkaline phosphatase activity was discovered in the walls of the blood capillaries, situated mainly around the periphery of the liver lobules. The enzyme was localized in the nuclei and in the cytoplasm of the endothelial cells. In individual cells of the endothelium of the septal veins, it was found in the form of traces and was localized to the nuclear nucleoli of the endothelial cells.

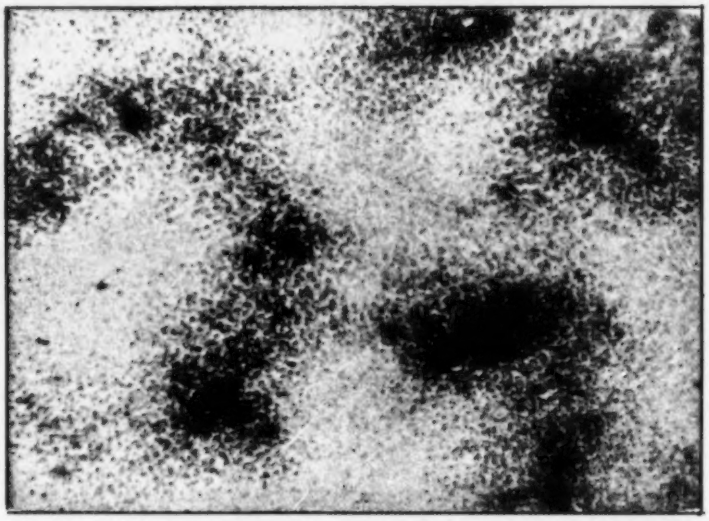

Fig. 3. Distribution of alkaline phosphatase in the liver of an irradiated rat. Treated by Gomori's method. Objective 8 ×, ocular 7 X.

Acid phosphatase was detected in the sections of the liver of the control animals in the nuclei of the liver cells and the endothelium of the blood capillaries, most of the enzyme being present in the nucleoli. Only traces of acid phosphatase were found in the epithelium of the interlobular bile ducts.

After irradiation for 6 days with the radioactive isotope of iron Fe^{59}, the following changes were found in the distribution of the enzymes in the liver. The alkaline phosphatase activity was increased in the nucleoli of the liver cells and the number of leucocytes had increased in the lumen of the blood capillaries with high enzyme activity. At the same time, in the wall of the interlobular bile ducts, alkaline phosphatase was found (Fig. 2). The enzyme was situated in all the cells of the wall, especially in the epithelial cells, and moreover alkaline phosphatase was found in both the nuclei and the cytoplasm of these cells. Sometimes in the films areas of intense blackening could be seen in the wall of the bile ducts, in which there were no visible structures. The liver cells bordering these areas, and also in places collections of leucocytes with very high enzyme activity showed a greatly increased content of the enzyme.

High activity of the enzyme was also found in the interlobular connective tissue of the liver, but its localization could not be determined, since all the cells of this tissue were blackened (Fig. 3).

No changes in the localization of acid phosphatase activity in the liver of the animals after irradiation, by comparison with controls, could be discovered.

The experiments thus showed that the alkaline phosphatase in the liver of control rats was found in the nuclei of the liver cells. The enzyme was localized in high concentration in the leucocytes and the wall of the blood capillaries. Acid phosphatase was found in the nuclei of the liver cells, the endothelium of the blood capillaries and the epithelium of the interlobular bile ducts.

After irradiation for 6 days with the radioisotope of iron Fe^{59}, increased alkaline phosphatase activity and an increase in the number of liver cells containing this enzyme were found.

The localization and activity of acid phosphatase remained visibly unchanged.

SUMMARY

The distribution of the acid and alkaline phosphatase was studied in the liver of white rats irradiated internally with radioactive iron Fe^{59}. For 6 days 25 – 45 μC per day of Fe^{59} was given with food (to experimental animals). The phosphatases were examined by the Gomori's method.

In control rats alkaline phosphatase was revealed in the nuclei of hepatic cells. High concentrations of the enzyme were noted in the leucocytes and the walls of the blood capillaries. The acid phosphatase was found in the nuclei of hepatic cells, endothelium of blood capillaries and in the epithelium of the bile interlobular ducts.

Following a 6-day irradiation with Fe^{59} an increase of the alkaline phosphatase activity along with the rise in the number of hepatic tissue elements containing it was observed. Acid phosphatase localization and its activity remain without any visible changes.

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PHARMACOLOGY

THE ACTION OF SOME INDUSTRIAL POISONS ON THE MECHANISM OF TRANSMISSION OF THE NERVE IMPULSE IN THE SUPERIOR CERVICAL SYMPATHETIC GANGLION

COMMUNICATION I. ACUTE EXPERIMENTS WITH HYDROGEN SULFIDE, ETHYLENE AND PROPYLENE ON HEALTHY ANIMALS

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The industrial poisons hydrogen sulfide and the unsaturated hydrocarbons ethylene and propylene are gases with which workers in the oil industry often have to come in contact during the manufacture of gasoline. Much work has been done to study the action of hydrogen sulfide and the ethylene hydrocarbons on the activity of the heart, the blood, gastrointestinal tract, eyes, hearing and nervous system; the subject of the effect of hydrogen sulfide and unsaturated hydrocarbons on the synaptic transmission of the nerve impulse, and in particular in the sympathetic nervous system, has been neglected.

We decided to try to rectify this omission, using the method of perfusion of the superior cervical sympathetic ganglion, developed by Academician K. M. Bykov and A. M. Pavlova [1] and by A. V. Kibyakov [2], and repeatedly commended both in the USSR and abroad.

EXPERIMENTAL METHOD

Experiments were carried out on cats, since they have a well-developed nictitating membrane, the contraction of which may be recorded on the smoked surface of a kymograph with a slowly revolving drum. After dissection of the superior cervical sympathetic ganglion, usually under urethane anesthesia (1 g/kg body weight, intraperitoneally), and setting up the system of perfusion of the ganglion with Ringer-Locke solution, the contractions of the nictitating membrane of the cat were recorded in response to stimulation of the preganglionic fibers by the electric current. The strength of the stimulating current was always 20 mm above threshold. Next, hydrogen sulfide, in a dose of 2 ml of a concentration of 0.1-1.0 mg/ml of Ringer-Locke solution, was injected by means of a syringe into the carotid artery, which supplies the perfused ganglion, and the contractions of the muscle of the nictitating membrane were again recorded in response to electrical stimulation of the preganglionic fibers with a current of the same strength.

EXPERIMENTAL RESULTS

The experiments showed that hydrogen sulfide, in a concentration of 0.2-0.5 mg/ml Ringer-Locke solution, caused excitation of the ganglion cells, and this excitation was removed by atropine added to the perfusion solution in concentration of $1:10^6$.

In higher concentrations (0.6-1.0 mg/ml Ringer-Locke solution), on the other hand, hydrogen sulfide has a depressing action on the superior cervical sympathetic ganglion, which proceeds along the lines of a Vvedenskii parabiosis. This effect can be abolished by cocaine; furing perfusion of the superior cervical sympathetic gang - lion with Ringer-Locke solution containing cocaine in concentration of $1:10^6$ the phenomenon of depression no longer develops in response to hydrogen sulfide.

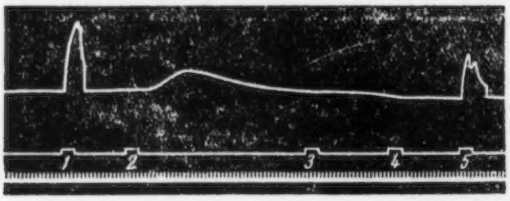

Fig. 1. The action of hydrogen sulfide on the humoral transmission of the nerve impulse in the superior cervical sympathetic ganglion.
Significance of the curves (from above down): contraction of the muscle of the nictitating membrane of the cat; stimulus marker and marker of injection of hydrogen sulfide and of "active perfusate" (1, 5 – response to electrical stimulation of the preganglionic fiber; 2, 4 – injection of 2.5 ml of "active perfusate" into the artery of the ganglion; 3 – injection into the artery of the ganglion of hydrogen sulfide in a concentration of 0.7 mg/ml Ringer-Locke solution, in a volume of 2 ml); time marker (5 seconds).

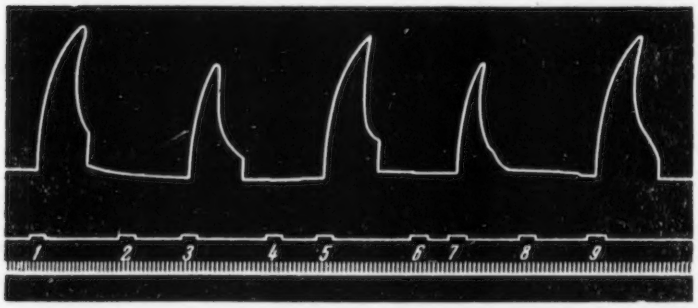

Fig. 2. Depressing action of hydrogen sulfide (concentration 0.8 mg/ml Ringer-Locke solution) on the superior cervical sympathetic ganglion and the restorative action of vitamins B_2 and C.
Significance of the curves (from above down): contraction of the muscle of the nictitating membrane of the cat; marker of stimulus and of injection of hydrogen sulfide and vitamins (1, 3, 5, 7, 9 – response to electrical stimulation of the preganglionic fiber; 2, 6 – injection of hydrogen sulfide into the artery of the ganglion; 4 – injection of vitamin B_2 in a dose of 2 ml of a concentration of 50 mg/100 ml Ringer-Locke solution; 8 – injection of vitamin C in a dose of 2 ml of a concentration of 50 mg/100 ml Ringer-Locke solution into the artery of the ganglion); time marker (5 seconds).

On the basis of these experiments we were able to state that hydrogen sulfide abolished primarily the humoral transmission of the nerve impulse, not the electrical (Fig. 1). If at first on the smoked surface of the kymograph drum are recorded the contractions of the nictitating membrane of the cat in response to electrical stimulation of the preganglionic fibers, and then in response to the injection of active perfusate* into the artery of the ganglion and we then inject into the artery of the ganglion hydrogen sulfide in a dose of 2 ml of a concentration of 0.7 mg per ml Ringer-Locke solution, then against the background of this poisoning of the ganglion, the contractions of the nictitating membrane of the cat from electrical stimulation of the preganglionic pathways occurs, although they are diminished, but the humoral transmission of the nerve impulse disappears completely.

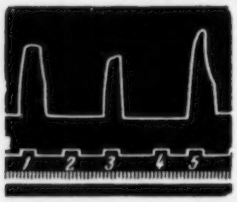

Fig. 3. The toxic action of propylene (concentration 0.4 mg/ml) and the restorative effect of vitamin B_6 on the superior cervical sympathetic ganglion. Significance of the curves (from above down): contraction of the muscle of nictitating membrane of the cat; marker of stimulus and of injection of propylene and vitamin B_6 (1, 3, 5 – response to electrical stimulation of the preganglionic fibers; 2 – injection of propylene into the artery of the ganglion; 4 – injection of vitamin B_6 in a dose of 2 ml of a concentration of 50 m/100 ml of Ringer-Locke solution); time marker (5 seconds).

A fact of great interest is the removal by vitamins B_2 and C of the depressing influence of hydrogen sulfide on the transmission of the nerve impulse in the superior cervical sympathetic ganglion. Vitamins B_2 and C not only abolish the toxic action, but if injected into the artery of the perfused ganglion before the action of hydrogen sulfide on it, in concentration of 0.6-1.0 mg/ml, they prevent the development of depression of the ganglion (Fig. 2).

In our later experiments to elucidate the action of unsaturated hydrocarbons on the synaptic transmission of the nerve impulse in the superior cervical sympathetic ganglion, we had the following aims:

1) To investigate what effect – depressing or exciting – was shown on the synaptic transmission of the nerve impulse in the superior cervical sympathetic ganglion by such products of oil refining as ethylene and propylene;

2) To ascertain precisely which form of transmission of the nerve impulse – humoral or electrical – was primarily affected; and

3) To select antagonists to neutralize the toxic action of the products of oil refining on the sympathetic nervous system.

Our experiments showed that ethylene injected into the artery supplying the ganglion in a concentration of 0.1 mg/ml Ringer-Locke solution and a dose of 2 ml, and propylene in a concentration of 0.4 mg/ml, have a slowly developing depressing action on the synapses of the sympathetic nervous system; under these circumstances propylene is more toxic than ethylene. Attention is drawn to the fact that the humoral transmission of excitation in the superior cervical sympathetic ganglion is suppressed primarly by these poisons as is also the case with hydrogen sulfide, and often it disappears completely, but the electrical conduction of the nerve impulse, although depressed, is nevertheless preserved (Fig. 3). During perfusion of the ganglion with Ringer-Locke solution containing cocaine in a concentration of $1:10^6$, this phenomenon of depression is absent. The disturbance of conduction of the nerve impulse through the synapse is also readily restored by injection of vitamins B_2, C and B_6 into the ganglion; vitamins B_1, PP and B_{12} do not possess this action.

In some experiments we found functional changes in the ganglion, which by their character were reminiscent of the equalizing and paradoxical stages of Vvedenskii.

* By active perfusate we imply the fluid collected from the internal vein of the sympathetic ganglion during stimulation of the preganglionic pathways by means of an electric current, and capable, on reinjection into the artery of the perfused ganglion, of causing contractions of the muscle of the nictitating membrane of the cat.

The excitant effect of hydrogen sulphide on the superior cervical sympathetic ganglion in cats may be removed by atropine, while the depressing one – by cocaine. Under the effect of hydrogen sulfide, ethylene and propylene the humoral transmission of the nerve impulse in the superior cervical sympathetic ganglion disappears earlier than the electric transmission. The functional changes produced as a result of the action of ethylene and propylene on the superior sympathetic ganglion are of the Vvedenskii parabiosis type. Vitamins B_6, B_2 and C remove the inhibitory effect of hydrogen sulfide, ethylene, and propylene on the superior cervical sympathetic ganglion of cats and render a protective effect. Vitamins B, PP, B_{12} lack such an effect. All experiments were conducted on healthy cats.

SUMMARY

Hydrogen sulfide has an exciting effect on the superior cervical sympathetic ganglion of cats in the concentration of from 0.2 to 0.5 mg/ml in Ringer-Locke solution and a depressive effect in the concentration of 0.6– 1.0 mg/ml in Ringer-Locke solution.

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THE GROWTH AND DEVELOPMENT OF BACTERIA IN CONNECTION WITH THE PHENOMENON OF BACTERIOPHAGE

COMMUNICATION VI. INVESTIGATION OF THE NUCLEAR STRUCTURES OF BACTERIA BY MEANS OF THE ELECTRON MICROSCOPE AND MICROCHEMICAL ANALYSIS

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In research on various aspects of the problem of the growth and development of bacteria in connection with the phenomenon of bacteriophage, the study of the influence of phage on the nuclear structures of bacteria is of fundamental importance.

As we know, the foundations of the study of the relationships between phage and the chromatin elements was laid as long ago as 1934 by N. N. Zhukov-Verezhnikov and V. A. Friauf [2]. In 1943-1946, their findings on the loss of basophilia of the protoplasm by bacteria under the influence of phage, together with a sharper delineation of the nuclei, were confirmed by M. A. Peshkov [5], Luria and Palmer [8], and subsequently by the observations of other workers.

In recent years papers have been published in which are given the results of electron microscopic investigations of the nuclear apparatus of bacteria under normal conditions and affected by phage. Since these investigations were carried out on a limited number of bacteria, and since the conclusions derived show certain contradictions, we considered it desirable to continue work in this direction by studying other species of microorganisms both under normal conditions and when affected by phage.

EXPERIMENTAL METHOD

The nuclear structures were studied by means of electron microscopy of cells from 18-20 hour broth cultures of Bact. coli aerogenes 1321 and of sections of these bacteria of varying thickness. The thicknesses of section mainly used were 250, 500, 750, 1000 and 1250 A, prepared with a Cambridge microtome.

In order to obtain ultrathin sections we used fixation and mounting of the bacteria in methacrylic resins according to the Birch-Andersen, Maale and Sjöstrand formula [7]. For this purpose, to 15 ml of an 18-20 hour broth culture of B. coli, diluted in the standard manner to $25 \cdot 10^4$ cells per ml, was added 1.5 ml of 2% OsO_4 in a buffer (a sterile solution of KH_2PO_4 1.45 g, $Na_2HPO_4 \cdot 2H_2O$ 6 g and NaCl 4.8 g in 1000 ml of distilled water). The mixture thus obtained was centrifuged for 5-6 minutes at a speed of 6000 rpm, after which the precipitate was resuspended in 1 ml of buffer and again mixed with 1 ml of 2% osmium tetroxide (OsO_4). The new mixture was incubated at 37° for 2 hours, during which time fixation was completed. After fixation, the bacteria were washed in 15 ml of buffer, and after centrifugation for a second time, they were resuspended in 70° ethyl alcohol, in which they were allowed to stand for 2 hours at room temperature (at 4° at night). The bacteria were then passed through 96° and absolute alcohol, and allowed to stand in each for 2 hours. After dehydration, the bacteria were transferred to 2 ml of a mixture of n-butylmethacrylate and methylmethacrylate (9:1) with 1% of benzoyl peroxide (as a

catalyst). The replacement of ethyl alcohol by the metacrylates enabled 4 successive transfers of the cells to be made at intervals of not less than 1 hour by means of centrifugation for 5-10 minutes at a speed of 6000 rpm. The final (fourth) volume of methacrylates, together with the suspended bacteria, was poured by means of a Pasteur pipette into dry No.2 gelatin capsules, which were incubated at 55-60° for polymerization, which was usually complete after 24-48 hours.

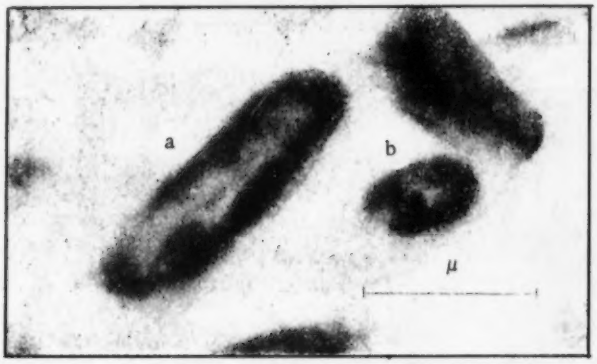

Fig. 1. Sections of Bact. coli aerogenes
a) Longitudinal section; in the center of the cell is a slender, broken up nuclear band; b) transverse section; in the center of the cell is the section of a round, nuclear band. Magnification 20,000 x.

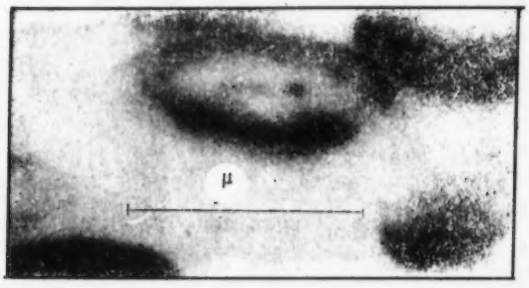

Fig. 2. Longitudinal section of Bact. coli aerogenes showing an elongated nuclear band. Magnification 20,000 x.

The prepared blocks were cut into sections by means of glass knives. The sections were assembled on copper gauze, coated with a collodion film, and the preparations were subsequently dried in air.

Electron-microscopic examinations of the intact bacteria and of their sections were supplemented by optic studies of stained preparations. For this purpose sections were transferred from the back of the knife to a drop of 20% alcohol on a cover glass. The sections were dried on the glass and then stained by the Romanowsky-Giemsa and Feulgen methods.

Each series of experiments thus consisted of an electron-microscopic study of the intact bacteria and of sections of these bacteria of varying thickness, supplemented by microchemical analysis of stained preparations.

EXPERIMENTAL RESULTS

Intact cells. On examination of intact cells of Bact. coli aerogenes under the electron microscope, no special features could be distinguished on the basis of which some idea could have been obtained on inclusions of

nuclear type in the protoplasm. The protoplasm of bacteria cultivated in meat-peptone broth and on meat-peptone agar was characterized in every case by its homogeneity, in the sense of its density in relation to a stream of electrons. Nor could light zones in the protoplasm, usually taken to be nuclei [6], be detected either during examination of preparations of bacteria which had been grown on a collodion film, i.e. in conditions which most favored the preservation of their natural form. In several experiments preparations of bacteria were examined from cultures after growth in broth for periods longer than 48 hours. The protoplasm of such bacteria was characterized by shrinkage and by general translucency, i.e. by a reduction of electron-optic density. Under these circumstances at the poles of many of the bacteria and sometimes in other areas too, electron-optically dense granules were found in the protoplasm, but these were not nuclei.

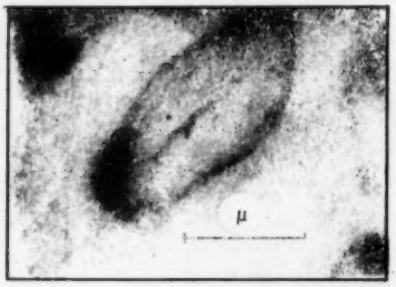

Fig. 3. Longitudinal section of Bact. coli aerogenes showing the nucleus and "polar cap." Magnification 20,000 x.

A study of the intact cells, stained as described above, by examination under the ordinary microscope, showed that certain formations were nevertheless present in the bacteria which, by their microscopic properties might be regarded as nuclear. In bacterial preparations stained by the Romanowsky-Giemsa method, these formations were distinguishable against the pale pink background of the protoplasm by virtue of their intense reddish violet staining. The majority of these had the appearance of structures stretched along the length of the cell. Some of them were characteristically round or spherical in shape, and in such cases 2-3 formations were present. In individual cells, at the poles, at the same time granules were found, which stained intensively a violet color, giving the appearance of polar caps and which were evidently identical with the electron-optically dense granules found under the electron microscope.

In preparations of bacteria stained by Feulgen's method, after fixation with Carnoy's fluid an analogous picture was observed. The field of vision of these preparations showed feebly stained bacilli of a lilac color, with nuclear elements buried in their cytoplasm in the form of reddish violet inclusions. These inclusions resembled spheres or short rods, lying longitudinally, transversely or obliquely in the cells. Under these circumstances a small number of cells were observed in which no reddish violet nuclear structures were present (the bacterial cadavers of M. A. Peshkov). On electron microscopic examination of sections of bacteria, usually in the field of vision were seen bacterial sections whose external form was determined by the direction of the section, i.e. by a mainly longitudinal, oblique or transverse direction.

The internal contents could be examined most completely in sections of bacteria in the longitudinal direction (Figs. 1, 2). In this case, if the anatomy of the bacteria was studied by examining the sections from the periphery toward the center, the following characteristic features could be made out. After the electron-optically dense bacterial wall came a layer of a less dense substance in which were seen some extremely fine granules and tubules between them. In the center of the section of the bacteria was a layer which was almost without density in relation to electrons. On photographic impressions it resembled a characteristic vacuole. Nuclear structures were included within this vacuole and had the appearance of an electron-optically dense band, stretched longitudinally along almost the whole length of the cell. In sections of many of the cells, besides nuclear structures it was possible to see also sections of polar cytoplasmic granules (Fig. 3). Nuclear structures were absent from some sections. Cells which were cut across in a stage of division usually had two bands, situated one in each half of the dividing cell.

On examination of the transverse sections of the bacteria the nuclear structures appeared as small, round formations, resembling bands transversely (see Fig. 1).

In oblique sections the nuclear structures had the appearance of short, round bands with margins cut across at an angle. Comparison of the external appearance of the nuclear structures in the sections in various directions showed that they were dense, round bands included in the zone of the substance which possessed almost no density in relation to electrons.

The external appearance and the character of the arrangement of the nuclear structures in the bacterial sections, as shown by the electron microscope, were analogous to the appearance and character of the nuclei revealed by microchemical analysis of the stained films.

The most suitable sections for staining by the Romanowsky-Giemsa method were those with a thickness of 1000 A. In longitudinal sections it was also easy to distinguish the cell wall, staining a violet color, and the cytoplasm, staining a barely perceptible rose-pink color, and against this background could be seen the outlines of the reddish violet nucleus, in the form of rods, with sometimes the presence of violet polar caps. In the more highly elongated cells, 2 nuclear inclusions were also found. In transverse sections of bacteria, stained by the Romanowsky-Giemsa method, nuclear formations were seen in the form of punctate violet inclusions.

As regards the sections stained by the Feulgen method, we were unable to produce satisfactory ones which showed the individual details with sufficient clarity. This was evidently due to damage sustained by the sections of the bacteria during the performance of the Feulgen reaction.

Certain authors are known to believe that the nuclear substance does not exist in bacteria in a discrete state, and workers who describe these nuclei usually have mistakenly interpreted the so-called polar caps [1, 3, 4].

Strong criticism of these views was expressed some time ago by M. A. Peshkov [6], who showed experimentally that nuclear substance is found in the form of aggregates in the cytoplasm of bacteria independently of the polar caps. Under these circumstances he emphasized that if the nucleoids were spherical in appearance, then polar caps were absent, and conversely, polar caps were always accompanied by rod-like nucleoids.

Our results also showed that the nuclear elements were present in the cytoplasm in a discrete form, independently of the presence of polar caps, which we called cytoplasmic granules, in accordance with the generally accepted terminology, since they might be more than two in number. In contrast to M. A. Peshkov, however, working with the optic microscope, on the basis of our electron microscopic investigations on a large number of sections, we came to the conclusion that the nuclear structures always had the appearance of longitudinal bands, corresponding to the rod-like nuclear elements observed during examination of the bacteria under the optic microscope. So far as the spherical nuclear structures described by many authors, including M. A. Peshkov, are concerned, we considered that their formation was associated with the different directions of the cells on the cover glasses when the films were fixed. In a cell fixed "square" on the glass, for example, the nucleoid was always spherical, just as took place in transverse sections of bacteria. Reports of the different shapes of the nucleoids are, evidently, the result of examinations of bacteria which were orientated differently in the plane of the film and, it appeared to us, do not reflect the true shape of the nuclear structures.

In conjunction with the data in the literature, our findings enable the general structure of the nuclear apparatus of bacteria to be judged to some extent. It may evidently be regarded as vacuoles, situated in the central part of the internal contents of bacterial cells, and consisting of material easily penetrated by electrons, in which are included dense bands, elongated in a longitudinal direction. These bands are possibly identical with the chromosomes.

In the structure of the nucleoids of bacteria further evidence may therefore be seen of their resemblance to the nuclei of the higher forms of cells, which agrees with the opinion of Piekarski and Giesbrecht [9] on the possibility of substituting the idea of "nucleus" for that of "nucleoid", and which indicates the chromosomal nature of the nuclear bands.

SUMMARY

The electron-microscopic and microchemical study of the ultrathin sections of B. coli has established the presence of discrete nuclear formation in the form of elongated oval bands. The nuclear formations being independent elements are not related to cytoplasmic granules ("polar caps") and do not change their morphology in the presence of the latter.

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THE REVERSION OF STABILIZED STRAINS OF L-FORMS OF PATHOGENIC STAPHYLOCOCCI

COMMUNICATION I. SPECIAL FEATURES OF THE PROCESS OF REVERSION

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The L-forms of bacteria are reactive formations which grow in the form of characteristic L-colonies in agar, having a characteristic morphological structure consisting of spheres, vacuoles and tiny granules, and sharply distinguishable from the original strains by their biological properties. The L-forms are extremely highly resistant to the action of the factors which were responsible for their formation. The process of formation of L-forms has been carefully studied in several bacterial species [6, 4, 1 and others]. Less attention has been paid to their biological properties [8, 5, 7, 1 and others], and there are only individual papers devoted to the study of the processes of reversion of L-forms back to the original strains [3, 4, 2].

In this connection it appeared to be of interest to obtain L-forms of the pathogenic staphylococci by means of the action of penicillin, and to study the laws governing the processes of reversion of these formations and the biological properties of strains undergoing reversion.

EXPERIMENTAL METHOD

L-forms of pathogenic staphylococci were obtained on 0.3% agar, prepared in Hottinger's broth with the addition of 20% of normal horse serum, 5% sodium chloride and various concentrations of penicillin (from 1 to 10,000 units/ml). In the same medium, but with higher concentrations of penicillin (from 1000 to 10,000 units/ml) successive passage (6-7 subcultures) of the strains of L-forms thus obtained was carried out in order to stabilize them in this state. Stabilization of the strains was proved by control experiments which demonstrated complete absence of viable cocci among the L-form colonies.

In order to obtain reversion it was necessary to provide conditions which would exclude the action of penicillin as a factor causing the formation and stabilization of L-forms, and would, at the same time, be favorable for the development of both L-forms and for the growth of staphylococci. These conditions were satisfied by 0.3% agar with 20% serum and 5% sodium chloride. On to this medium were subcultured strains 75L, 5L, 8HL, Lossmanov-L (after 6-7 passages through a medium containing penicillin) and 75L (after 13 passages through a medium with penicillin). In order to procure reversion, passages of the strains of L-forms were made every 14 days, during which time the cultures were kept continually at a temperature of 37°. Before every serial passage a note was made of the character of growth, and phase-contrast microscopy of the colonies was carried out. At the same time as each subculture into serum-salt agar was made, a control inoculation was performed into Hottinger's broth to which was added 1% glucose and 5% serum. Growth of cocci in these tubes was shown only after reversion of the L-forms in the serum-salt agar. Passage through a penicillin-free medium was continued for 5 months, and altogether 10 passages were made.

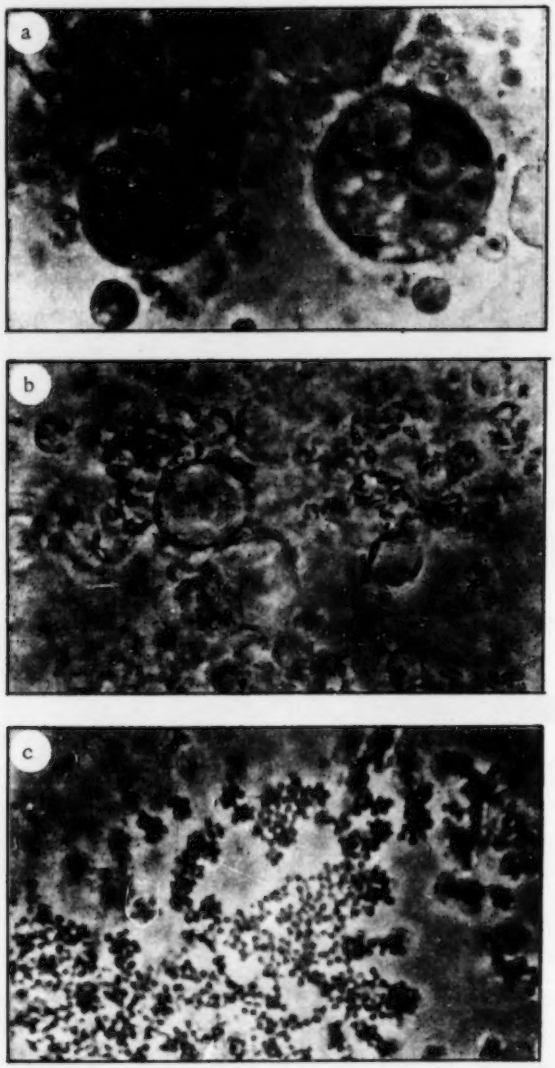

Fig. 1. Stages in the reversion of stabilized cultures of L-forms of pathogenic staphylococci. Phase-contrast microscopy. Objective 90 x, ocular 15 x.
a) Giant spheres with marked vacuolization. Separate granular forms; b) vacuoles with thin walls, inside which are inclusions of various sizes and shapes; c) pure culture of staphylococci, reverting from a stabilized culture of L-forms.

EXPERIMENTAL RESULTS

Of the five strains of L-forms of pathogenic staphylococci selected for the experiment, reversion was obtained in three – 75L (previously subcultured on 7 occasions into medium containing penicillin), 5L, and Lossmanov-L. The two remaining strains – 8HL and 75L (after 13 passages through medium with penicillin) retained their ability to develop as L-forms even after passage for 5 months in the absence of antibiotic.

Analysis of the results given in the table shows that the intensity of the reversion process depended, on the one hand, on how deeply stabilized was the strain of the L-form, and on the other hand, on the individual properties of the strain of staphylococcus used for production of the L-forms.

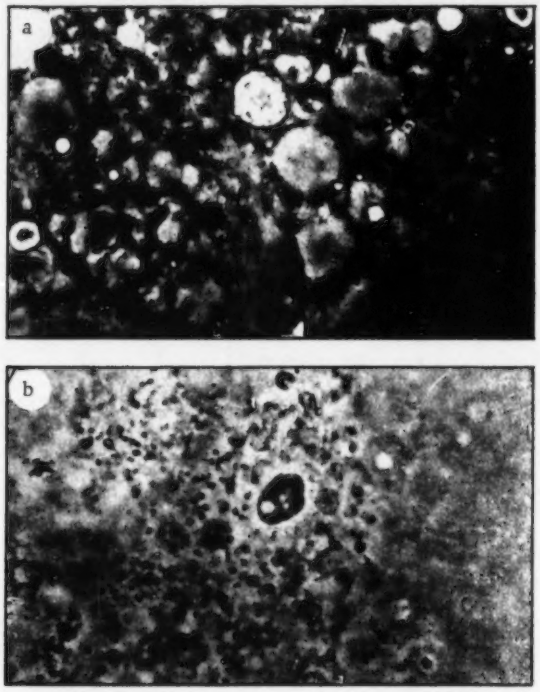

Fig. 2. Stages in the reversion of stabilized cultures of L-forms of pathogenic staphylococci.
a)Clear, light-refracting bodies among spheres, vacuoles and granular elements of L-forms. Magnification × 1350; b) area of large granules, approximately resembling staphylococci in their external appearance. Magnification × 1350.

In confirmation of the first statement was reversion of the cultures of strain 75L. Although obtained from the same strain, these cultures behaved differently in the conditions bringing about reversion. A culture of 75L, used in the experiment after 7 passages on agar containing penicillin, reverted at the 3rd subculture, whereas a culture of 75L, after 13 preliminary passages on a medium containing penicillin, showed no reversion to staphylococci after 10 subcultures in corresponding conditions.

The second statement follows from the course of reversion of cultures of strains Lossmanov-L and 8HL. Despite the fact that these strains, in contrast to 5L and 75L (7th passage) had been subcultured only six times on medium containing penicillin, they showed considerable resistance to reversion on medium without penicillin. Strain Lossmanov-L reverted at the 8th passage, but strain 8HL preserved all the properties of the L-forms up to and including the 10th passage.

The course of the morphological changes observed during passage of stabilized cultures of L-forms on a medium not containing penicillin, and during reversion of L-forms into the original strain, showed highly distinctive features in the individual strains. Depending on the character of these changes, all the strains used in the reversion experiment could be divided into three groups.

Reversion of Stabilized Cultures of L-Forms of Pathogenic Staphylococci during Passage on Serum-Salt Agar without Penicillin

Strains selected for the experiment: L-forms		Time of passage									
		1-month		2-months		3-months		4-months		5-months	
Name of strain	Number of preliminary passages on medium containing penicillin	1st passage	2nd passage	3rd passage	4th passage	5th passage	6th passage	7th passage	8th passage	9th passage	10th passage
75 L	7	L-form	L-form	reversion							
5 L	7	L-form	L-form	reversion							
Lossmanov L	6	L-form	L-form	L-form	L-form	L-form	L-form	L-form	reversion		
8HL	6	L-form	L-form	L-form	L-form	L-form	L-form	L-form	L-form	L-form	L-form
75 L	13	L-form	L-form	L-form	L-form	L-form	L-form	L-form	L-form	L-form	L-form

The first group included strains 75L (7th passage) and 5L. In the first days of development on a medium without penicillin, these cultures grew with the appearance of typical L-forms, but marked vacuolization began to show itself as giant forms, up to 25-30 μ in diameter and with the development of granularity (Fig. 1, a). At the end of the second passage, the colonies consisted entirely of large (from 5 to 15 μ) vacuoles with thin walls, inside which were round and elongated inclusions, varying in thickness (Fig. 1, b). At the beginning of the 3rd passage the number of inclusions as described above, and of small granular formations increased. Such a picture immediately preceded the discovery under the microscope of proliferating cocci, a pure culture of which is shown in Fig. 1, c, after isolation on an agar plate.

The second group consisted of strain Lossmanov-L. During the first passages on medium without penicillin, this strain did not differ in its morphology from typical cultures of L-forms, but at the 3rd passage there appeared numerous clear, light-refracting bodies, the number of which increased sharply in the 5th, 6th and 7th passage (Fig. 2, a). At this time there appeared large areas of coarser granules, resembling cocci in their outward appearance (Fig. 2, b). A similar picture immediately preceded the appearance of coccal forms.

The third group included strains 8HL and 75L (13th passage). Throughout all 10 passages these strains grew readily on serum-salt agar with the appearance of typical L-forms. Only at the 9th-10th passage of strain 75L (13th passage) was the appearance of numerous vacuoles observed, with thin walls and inclusions of various sizes and shapes.

Stabilized cultures of L-forms of pathogenic staphylococci thus possessed the ability to undergo subculture for $1\frac{1}{2}$ to 5 months on serum-salt agar without penicillin as L-forms. The rate of reversion depended on the duration of preliminary passage of the culture of L-forms in the presence of penicillin and on the individual properties of the original strain. Significant differences were observed in the morphology of the various stages of reversion of the individual L-form cultures.

SUMMARY

The author studied the process of reversion of the stabilized cultures of the L-forms of pathogenic staphylococci under the effect of penicillin. The passage of the L-form stabilized cultures on the serum-salt agar without penicillin was possible for from $1\frac{1}{2}$ to 5 months. The rate of the reversion process depended on the duration of the passage of the L-form in the presence of penicillin, and on the individual properties of the initial strain. Significant variations were observed in the morphology of individual stages of reversion of different L-form cultures.

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A STUDY OF THE POSTVACCINATION IMMUNITY OF GUINEA PIGS REPEATEDLY INOCULATED WITH A STRAIN OF BRUCELLA MELITENSIS

COMMUNICATION II.

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Many years of research by P. F. Zdrodovskii, B. V. Voskresenskii, P. A. Vershilova, V. A. Shtriter, Kh. S. Kotlyarova and others [1-5] on the reproduction of experimental brucellosis in different species of animals and on studies of aspects of pathogenesis and immunity of this condition laid the foundations of the scientific development of methods of active prophylaxis of infections caused by these microorganisms.

Work by P. A. Vershilova proved that the immunological basis of the guinea pig could be modified by the subcutaneous injection of living vaccine from the strain of Br. abortus 19-BA, using a dose of 10^9 organisms.

A result of the use of living vaccine from strain BA, a marked decrease in the incidence of the disease has been achieved among persons working in areas of brucellosis among sheep. Moreover cases are known where persons vaccinated with living brucellosis vaccine have developed the disease after meeting large doses of the causative organism; under these circumstances the incidence of the disease among those vaccinated varied from 0.5 to 6-8%.

Every case of breakdown of immunity is known to be due, as a rule, to the relative nature or to the weak intensity of postvaccinal immunity. It may be considered that in both the phases of sterile and unsterile immunity, its intensity in reasonably identical conditions of vaccination may differ, depending on the individual susceptibility of the host on the one hand, and on the character and the frequency of super- and reinfection on the other. In this connection the study of the possible conditions for breakdown of immunity at different stages of vaccination becomes extremely important. To this end we carried out investigations to determine the reaction of immune guinea pigs to repeated inoculations of virulent organisms of the brucella group, in the phase both of unsterile [6] and of sterile (postvaccination) immunity.

EXPERIMENTAL METHOD

Guinea pigs, weighing 350-400 g (50 in number) were inoculated subcutaneously in the right inguinal region with a dose of 10^9 organisms of a vaccine strain of Br. abortus 19-BA, suspended in 1 ml of physiological saline. $7\frac{1}{2}$ months after vaccination, these guinea pigs, in a state of sterile immunity, were given three injections (at monthly intervals) of a virulent strain of Br. melitensis 548, in a dose of 20 organisms, subcutaneously in the right inguinal region. The intensity of the sterile (postvaccinal) immunity was studied in 3 subgroups of immunized guinea pigs.

The first subgroup of animals (10 in number) were autopsied 30 days after the first inoculation, and their organs (lymphatic glands, liver, spleen, brain, bone marrow, heart muscle, blood and urine) were used to inoculate culture media. The guinea pigs of the second and third subgroups were reinoculated at monthly intervals

after the primary reinoculation, with Br. melitensis 548 in a dose of 20 organisms, given subcutaneously in the right inguinal region.

One month after the second reinoculation, the 10 guinea pigs of the second group were autopsied, and bacteriological examination of the organs was carried out. The animals of the third subgroup were reinoculated three times (at intervals of one month), and 30 days after the third reinoculation with the same strain in a dose of 20 organisms these also were autopsied, and their organs used to inoculate culture media. As a control of each group, autopsy was carried out at the same times on guinea pigs which had simply been vaccinated as well as those subjected to one and two reinoculations.

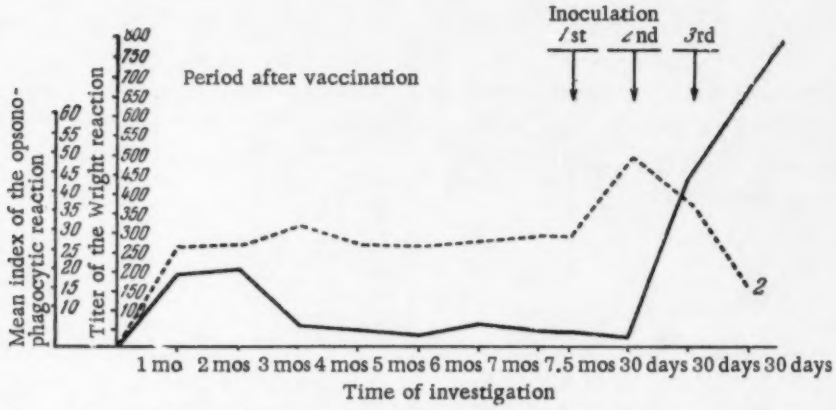

Fig. 1. Course of the Wright and opsono-phagocytic reactions in vaccinated guinea pigs during repeated reinoculation with a strain of Br. melitensis. 1) Wright reaction; 2) index of the opsono-phagocytic reaction.

Time since vaccin. at the time of inoc.	Number of times innoculated	Results of autopsy 30 days after inoculation
7½ months	Once	
	Twice, at monthly intervals	
	Three times, at monthly intervls.	
Control of vaccine strain		
Control of inoculation with Br. melitensis		

■ 1 ◧ 2 ◩ 3 □ 4

Fig. 2. Results of bacteriological investigations of immunized guinea pigs, reinoculated once, twice and three times with a strain of Br. melitensis. 1) Generalized infection; 2) infection of lymphatic glands; 3) regional infection; 4) culture from excreta.

Furthermore, besides bacteriological examinations, the agglutinin content of the animals was determined by Wright's reaction, together with the opsono-phagocytic reaction at the end of each month during vaccination (1 to 7 months) and also 30 days after each reinoculation, i.e. before autopsy of the animals. The mean values of the results obtained at each period of time in 20-25 immunized guinea pigs were calculated. Burnet's intradermal allergic test was carried out by the usual method at an interval of 30 days after vaccination and after each reinoculation.

EXPERIMENTAL RESULTS

The investigations during vaccination and repeated reinoculation with one, two or three doses of the organism in the phase of postinfective immunity gave the following results.

As can be seen in Fig. 1, in the guinea pigs immunized by inoculation of vaccine strain BA in a dose of 10^9 organisms showed characteristic immunological changes. The accumulation of agglutinins in the blood and the increase in the index of the opsono-phagocytic reaction began to take place on the 15th day after vaccination and reached their maximum after 2 months. After the third month, the trend of the Wright reaction and the opsono-phagocytic reaction in the vaccinated groups of guinea pigs showed a tendency to fall. At the moment of reinfection, i.e. $7\frac{1}{2}$ months after vaccination, the average titer of the Wright reaction and the opsono-phagocytic index reached their lowest values; 2 months after vaccination the Burnet intradermal allergic reaction was positive in the overwhelming majority of guinea pigs (25 of the 45 examined).

The Burnet Intradermal Allergic Reaction in Guinea Pigs at Different Intervals after Vaccination

Time after vaccination, months	Number of guinea pigs tested	Degree of the Burnet reaction					
		–	±	+	++	+++	++++
1	40	19	4	15	2	–	–
2	45	20	8	10	6	1	–
3	13	10	–	2	1	–	–
4	26	7	4	10	3	2	–
5	28	3	0	11	9	3	2
6	29	7	2	4	11	5	–
7	14	4	2	4	1	3	–

Note: Negative –; doubtful ±; weakly positive +; positive ++; markedly positive +++; strongly positive ++++.

It should be pointed out that in the control group of vaccinated guinea pigs, subjected to bacteriological examination, the vaccine strain was found up to 3 months after vaccination; on and after the fourth months after vaccination in no case were we able to isolate the vaccine strain from the animal's organs. Being satisfied in this manner that the vaccinated guinea pigs had passed both the phase of unsterile and the phase of sterile immunity, we began to study the role of repeated inoculation on the breakdown of postinfective immunity.

The results of the bacteriological examination of the organs of guinea pigs reinoculated on one, two or three occasions are illustrated by the data shown in Fig. 2, from which it can be seen that these results differed according to the number of reinoculations.

In the first group of vaccinated animals, for instance (10 in number), which received only one infecting dose, a strain of Br. melitensis was isolated from the regional lymphatic gland of only 2 guinea pigs, whereas in the remaining 8 animals (80%) no disturbance of the sterile immunity was observed. In contrast to this, in the experiments in which reinoculation was repeated twice at intervals of one month, the results of bacteriological examination revealed a breakdown of immunity in the majority of the experimental animals, i.e. Br. melitensis was isolated from 7 of the 10 guinea pigs, and moreover in 5 animals a generalization of the infection was found; in one a strain of Br. melitensis was isolated from many of the lymphatic glands, in the other – only from the regional lymphatic gland. Despite the two reinoculations, three guinea pigs of this subgroup preserved their insusceptibility.

The guinea pigs of the third subgroup, exposed to three infecting doses (at monthly intervals), were all found to be infected on bacteriological examination. Of the 10 guinea pigs in this subgroup, in 5 a generalized infection was demonstrated, in four Br. melitensis was isolated from the lymphatic gland, and in one guinea pig only the infection was regional in character. Further, at autopsy on 2 guinea pigs inoculated 3 times, the inguinal and cervical lymphatic glands were found to be enlarged to the size of walnuts, and contained pus. Subcultures taken from the pus from these guinea pigs also gave positive results of isolation of Br. melitensis.

The immunological reactions both during vaccination and, more especially, after repeated infection, showed very characteristic changes. In particular, as seen in Fig. 1, the mean titer in the Wright reaction and the mean index of the opsono-phagocytic reaction were characterized during the course of vaccination by a gradual rise, reaching a maximum at the end of the second month. From the third month after vaccination a gradual fall was observed in the blood agglutinin titer, and 7-8 months after vaccination the mean titer of the Wright reaction was 1:33, and the mean index of the opsono-phagocytic reaction throughout the process of vaccination, if we disregard slight variations, remained on the whole at roughly the same level (20-28). Of special interest were the fluctuations of the mean agglutinin titer and of the index of the opsono-phagocytic reaction in response to repeated infection. Thus it may be seen from Fig. 1 that whereas $7\frac{1}{2}$ months after vaccination and 30 days after the first inoculation the mean titer of the Wright reaction was 1:20 and the agglutinin titer was not increased, despite the inoculation of a virulent strain, soon after the second inoculation the trend of the Wright reaction altered sharply, on account of a rapid accumulation of agglutinins; on the 30th day after the second inoculation the mean titer (which was 1:20 after the first inoculation) reached 1:450.

Moreover, in guinea pigs receiving three inoculations, the accumulation of agglutinins on the 30th day after the third inoculation reached on the average the value of 1:750, i.e. almost 40 times greater than the original figure.

Analysis of the results obtained on the accumulation of agglutinins in the blood, by comparison with the results of bacteriological investigations of each guinea pig taken individually, revealed a characteristic picture: a close connection was observed between the accumulation of agglutinins and the degree of dissemination of organisms throughout the organs of the guinea pig. In the animals of the first subgroup, for instance, receiving one inoculation, the titer of the Wright reaction stayed at the same level both before and after infection, whereas during a generalized infection the mean titer of the agglutinins reached 1:1000. On the other hand, in guinea pigs from which strains could be isolated only from the lymphatic glands, the mean titer was 1:550, and in the presence of regional infection – 1:30.

By comparison of the results of the Wright reaction and the mean index of the opsono-phagocytic reaction in all 3 subgroups of guinea pigs it was shown that after repeated infection the titer of the Wright reaction rose and, on the contrary, the index of the opsono-phagocytic reaction fell. In guinea pigs with a low agglutinin titer and in case of regional infection the index of the opsono-phagocytic reaction was high (50-60), but in guinea pigs with an average Wright reaction titer of 1:1000, in the presence of generalized infection the index of the opsono-phagocytic reaction fell to 15 units.

A study of the trends of the Burnet intradermal allergic test also showed a reaction characteristic of the immunological reorganization of the animals during the process of vaccination and after each repeated inoculation. As may be seen from the table, in the fifth month after vaccination, in the overwhelming majority of the guinea pigs (in 25 of 28) the intradermal allergic reaction was positive. After the sixth month the intensity of the Burnet reaction gradually fell and in 7 of 29 guinea pigs at this time it was negative. The character of the intradermal allergic test in the vaccinated guinea pigs $7\frac{1}{2}$ months after vaccination, i.e. before the first inoculation, was shown by total extinction in 4 guinea pigs, by a weakly positive reaction in 2 and by a positive reaction in only 4 guinea pigs.

In the guinea pigs of the same subgroup, even a single superimposition of the infective agent, i.e. infection with Br. melitensis $7\frac{1}{2}$ months after vaccination had a strongly sensitizing effect. Only 30 days after the single inoculation the Burnet reaction became positive in all 10 guinea pigs. In the majority of the animals (in 6 of 10), 30 minutes after the single inoculation a positive Burnet intradermal reaction was noted, with an area of edema measuring 4 × 5 cm, and with tissue necrosis. In the remaining 4 guinea pigs the reaction was positive. Subsequently, in the guinea pigs subjected to two and three inoculations, an increase in the intradermal allergic reaction was observed also, with the development of tissue necrosis.

It may thus be seen from the results described that frequent superimposition of infection in the form of single, double or triple inoculation led to a severe allergic reorganization of the animal.

As a general conclusion from the results it may be said that the intensity of immunity in guinea pigs vaccinated with strain 19-BA of Br. melitensis is high in relation to a single reinfection during the period of sterile immunity.

The results obtained during double and triple reinfection indicate a comparatively rapid breakdown of the resistance of the vaccinated animal. This breakdown of immunity in 70% of guinea pigs after double reinfection and in 100% after triple (in contrast to the first subgroup of guinea pigs, in which 80% of the animals were insusceptible after a single reinfection) justifies the provisional hypothesis that such a breakdown of immunity is possible in vaccinated animals not only as a result of an encounter with a large dose of Br. melitensis, but also as a result of repeated encounters with small doses of infection.

SUMMARY

The "break" of vaccinal immunity both in the steril and nonsterile phases led the authors to perform the following experiments: the vaccinal process was experimentally induced in guinea pigs by administration of the 19-BA, vaccine strain. The intensity of the sterile (postvaccinal) immunity was studied by repeated infection with a virulent Br. melitensis culture. It has been established that 17½ months after immunization in 80% of cases the guinea pigs were still immune to a single infection with Br. melitensis. In reiterated infection with one month interval broken immunity was recorded in 70% of the cases, while a three-fold succession of infection with the same time interval – showed disrupted immunity in nearly 100% of the cases and a subsequent development of a generalized infection.

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AN INVESTIGATION OF PURIFIED TETANUS TOXOID BY THE METHOD OF PRECIPITATION IN AGAR

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The method of precipitation in agar has been widely adopted in recent years for the analysis of preparations of various antigens and immune sera.

In the present investigation we used this method for the study of purified tetanus toxoid.

EXPERIMENTAL METHOD

Tetanus toxoid, prepared in Ramón's medium, was purified and concentrated by a method devised by ourselves and described earlier [1], consisting of two main stages: precipitation with sulfosalicylic acid and dialysis in a collodion bag. The preparation was further purified in the cold (–10°) by precipitation with ethyl alcohol and subsequent centrifugation. Alcohol was added until its concentration was 55%. The mixture was allowed to stand for 50 minutes, after which the precipitate was removed by centrifugation and dissolved in a phosphate buffer (pH = 7.4), and the concentrate thus obtained was then dialyzed in a cellophane bag against tap water.

Titration by a standard method in white mice showed that the preparation contained 1300 units of combination (CU) per ml. Its specific activity was 1680 CU per mg nitrogen.

This preparation was subjected to further fractionation by gradual salting out with a phosphate mixture (pH = 7.4), containing 83.3% of dipotassium hydrogen phosphate K_2HPO_4 and 16.7% of potassium dihydrogen phosphate KH_2PO_4 [4]. The fractionation was carried out as follows. To a measured sample of toxoid was added a weighed portion of the phosphate mixture, and the whole was mixed and allowed to stand for 18 hours at 18°. The precipitate was collected on filter paper and dissolved in distilled water. To the filtrate was then added the next portion of phosphate mixture, and so on. Altogether 7 fractions were obtained.

EXPERIMENTAL RESULTS

As may be seen from the table, the bulk of the antigen (77%) was salted out between the limits of 0-25% of phosphate mixture. The fractions "15-20" and "20-25" showed the highest activity.

In order to carry out the precipitation reaction, we used the method of counterdiffusion (a modification of Ouchterlony's method [3]).

The agar was cleared in the following manner [2]: it was dissolved in 20 parts of distilled water and to it was added a 20% solution of calcium chloride to give a concentration of 0.5% after coagulating, the agar was cut up finely and washed for 3 days in tap water. The washed agar was melted, mixed with 2 volumes of a hot 1.15% solution of sodium chloride and filtered through cotton wool, after which 0.02% of merthiolate was added.

The agar, prepared in this manner, was poured in a layer of 3 mm into a rectangular dish which was placed perfectly horizontally. The dish consisted of a glass plate, to the edges of which pieces of glass tubing were secured by means of plastic glue.

Using a stencil, rows of small holes were cut in the layer of solidified agar, 15 mm in diameter and 10 mm from each other. On both sides of these holes, parallel to them and at a distance of 15 mm, grooves were cut at right angles to each other, 5 mm wide. Into the holes was poured 0.5 ml of the toxoid preparation to be tested, and into the grooves a tetanus antiserum, purified and concentrated by the Diaferm-3 method, of two different series. The dish was covered with glass and allowed to stand at room temperature for 10 days.

Characteristics of the Fractions of Purified Tetanus Toxoid Obtained by Salting Out with a Phosphate Mixture (pH = 7.4)

Percentage of phosphate mixture	Titer (in CU/ml)	Yield of antigen (in %)	Specific activity (in CU/mg of nitrogen)
0 - 15	400	10.3	1 270
15 - 20	1 300	33.3	3 300
20 - 25	1 300	33.3	3 270
25 - 30	250	6.0	428
30 - 35	100	2.0	510
35 - 40	< 100	—	—
40 - 45	< 50	—	—

When the reaction was performed by the method described, precipitation lines were formed in the layer of agar in the shape of an arc. If neighboring holes contained common antigens, the respective arcs merged together into a continuous, undulating line. Where the antigen which was contained in the other preparations being tested at the same time in neighboring holes was either absent or present in too low a concentration, the precipitation lines were interrupted.

In order to photograph the precipitation lines we used the contact method, using contrat paper for reflex printing. To remove spots in the dish before filming, a thin layer of water was poured on.

As may be seen from the photograph shown (Fig. 1), the purified toxoid formed 3-4 lines. It should be mentioned that in experiments performed at the same time, the number of lines may vary slightly as a result of more or less perfect separation of the lines, especially of those in very close contact with each other.

It was not possible to determine from the intensity of the precipitation lines which of them was due to the specific antigen – to the toxoid itself – since the degree of purification of the preparation was relatively low and some of the ballast antigens present could exceed the concentration of the specific antigen.

We were able to distinguish the line corresponding to the specific antigen by comparing the immunograms of the original, purified toxoid and its fractions, taking into consideration the results of estimations of the antitoxin combining power in white mice.

Let us compare the immunograms of two preparations: purified toxoid (No. 47) and fraction "20-25" (No. 46). According to the results of the biological experiment, both these preparations contained considerable and equal amounts of specific antigen (1300 CU/ml). Theoretically, in this case, we should have expected to see a continuous line of moderate intensity, due to the specific antigen, the arcuate portions of which would have been arranged symmetrically in relation to the corresponding holes in the agar. The lines closest to the holes, for example, conform to these requirements. If it is formed by the specific antigen, it must not be present, therefore, in the immunograms of the fractions not containing this antigen or containing it in too low a concentration.

In fact, in the immunograms of fractions "30-35" (No.51), "35-40" (No.53) and "40-45" (No.56), this line was absent. At the same time these fractions, as can be seen from the photograph, contained other antigens, presumably ballast, which were detected also in the original purified toxoid. In one fraction ("30-35") there were two such antigens, and in the others ("35-40" and "40-45") – predominantly one.

As an additional criterion of differentiation of the precipitation line of the specific antigen it was possible to make use of its relatively higher thermolability. It was shown by preliminary experiments on white mice that tetanus toxoid almost completely lost its antitoxin combining power after being heated for 20 minutes at 80°.

On the basis of these observations it might have been thought that a preparation treated in this way would not give a specific line of precipitation. In fact this line, clearly marked on the immunogram (No.46) of the "20-25" fraction, was absent from the immunogram (No.45) of the same fraction after heating, whereas the second line, corresponding to some other antigen, was clearly shown.

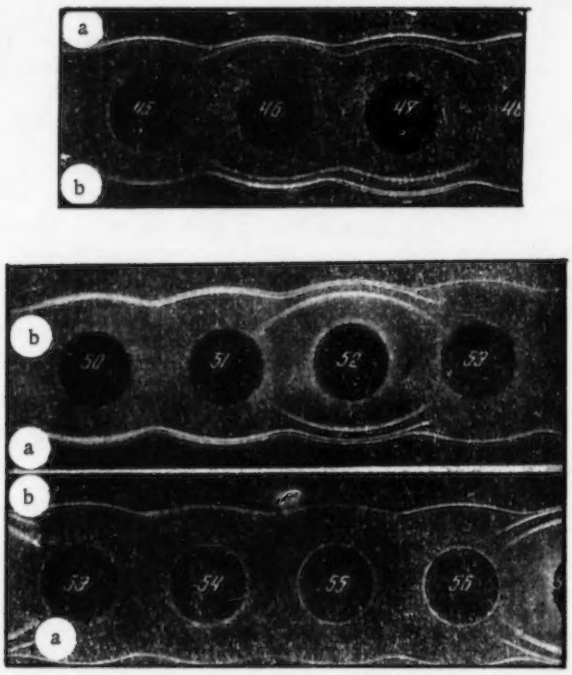

Fig. 1. Precipitation in agar of purified tetanus toxoid (series 1) and of certain of its fractions obtained by salting out with a phosphate mixture (pH = 7.4).
47 and 52) Original purified toxoid; 46 and 45) "20-25" fraction, before and after heating; 53 and 54) "35-40" fraction, before and after heating; 56 and 55) "40-45" fraction, before and after heating. Experiment carried out with tetanus antisera from horse, purified by the Diaferm-3 method, of series 438 (titer 2000 antitoxin units) and series 436 (titer 1400 antitoxin units). a) Serum 438; b) serum 436.

From the results of the precipitation reaction in agar, not all ballast antigens showed equal thermostability. After being heated in the manner described above, certain of them underwent some degree of damage. This was shown by the weakening of the corresponding precipitation line, or by its greater diffusion (immunograms Nos. 50, 54 and 55).

We give for comparison a photograph of the immunogram (Fig. 2) of purified tetanus toxoid of the second series, before and after heating. This preparation contained 4200 CU/mg of nitrogen.

The photograph clearly shows the disappearance of one of the precipitation lines as a result of heating the toxoid, whereas the other lines were preserved to a greater of lesser degree.

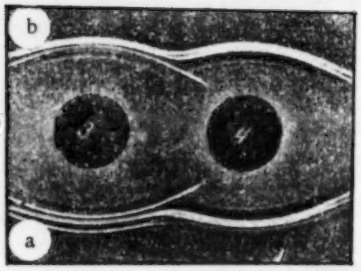

Fig. 2. Precipitation in agar of purified tetanus toxoid (series 2).
3)Original purified toxoid; 4) the same toxoid, heated for 20 minutes at 80°. a) Serum 438; b) serum 436.

Two of these lines, as may be seen from the immunogram, obtained by means of serum of series 438, appeared to split up into two diverging branches at one point.

This interesting phenomenon may possibly be accounted for by the formation of two forms of the same antigen, differing slightly in their physicochemical properties as a result of partical denaturation by heating.

The results described in this paper show that the method of precipitation in agar, when combined with other methods, may be used with success to investigate the antigenic composition even of such complex preparations as purified tetanus toxoid.

SUMMARY

Several antigens were detected in the purified tetanus antitoxinby the method of precipitation in the agar. These antigens gave different distribution in the fractions obtained by fractional precipitation of this antitoxin by the phosphate mixture (pH = 7.4). Precipitation of specific antigen (antitoxin) was differentiated by comparison of the data obtained by reaction of precipitation in the agar with the data of antitoxin-fixating reaction obtained in experiments on white mice. Relatively greater thermolability of the specific antigen compared with the admixed ballast antigens served as an auxiliary factor in this test.

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ONCOLOGY

THE ANTIGENIC PROPERTIES OF THE RED CELLS IN LEUKEMIA*

COMMUNICATION I. DIFFERENTIATION OF THE ANTIGENIC PROPERTIES OF NORMAL AND LEUKEMIC RED CELLS BY THE SPECIFIC DELAY OF PRECIPITATION TEST IN AGAR USING ANTISERUM AGAINST LEUKEMIC SPLENIC TISSUE

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We showed in previous research that tissues with leukemic infiltration differ in their antigenic properties from the corresponding normal tissues. Antigenic differences between leukemic and normal tissues could be found both by the anaphylaxis with desensitization test [2, 3] and by the specific delay of precipitation test in agar [4, 5].

Proliferating leukemic cells may be found not only in the tissues but also in the blood stream of patients with leukemia. Products of destruction of these cells may also enter the blood stream.

There are reports in the literature of patients with leukemia developing an autoimune hemolytic anemia [7, 8, 10 and others]. The factor responsible for modifying the antigenic properties of the red cells and causing autoimmunization is still unknown [6].

Assuming that the antigenic properties of the red cells are altered by adsorption of products of destruction of leukemic cells with a definite antigenic specificity, we compared the antigenic properties of normal and leukemic red cells, using antiserum to leukemic splenic tissue for their differentiation. The antigenic properties of the red cells were studied by the Ouchterlony specific precipitation in agar test [7] and by the specific delay of precipitation test.

EXPERIMENTAL METHOD

The precipitation test was carried out at room temperature in Petri dishes with a diameter of 96 mm. The dishes were filled with 1% transparent agar containing 0.8% NaCl and a preservative – methiolate (1:10,000). The volume of agar poured into the dishes was 24 ml (7 ml for the bottom layer and 17 ml for the top). The antigens and antiserum were poured into holes made in the top layer of agar. The results were read on the next day and again on the 2nd-4th day.

The antisera used in the test were obtained by immunization of rabbits with antigens from the splenic tissue of persons dying from leukemia or from trauma. The immunizing antigens consisted of a saline extract of the tissues of the whole spleen, of the hyaloplasm fraction and a nuclear fraction. The hyaloplasm fraction was obtained by removal of the nuclei and the cytoplasmic granules from the homogenate of splenic tissue by centrifugation for 1 hour at 20,000 rpm, and the nuclear fraction by the method of Schneider and Petermann [10]. The rabbits were immunized with an additive as suggested by Freund. 4 cycles of immunization were carried out. Each cycle consisted of 4 injections at 5-day intervals: 1st injection – 33 mg of antigen protein with addi-

* The subject matter of this article formed part of a lecture given at the 36th Plenum of the Central Order of Lenin Institute of Blood Transfusion on June 3, 1957.

tive (lanolin + vaseline with killed BCG) by Freund's method, the 2nd, 3rd and 4th injections without additive. The interval between the cycles was 6 weeks. Blood was taken in rising doses of antigen – 66, 99 and 132 mg – on the 7th, 14th and 21st day after the conclusion of the immunization cycle.

Antigens for the test were prepared from the red cells of patients with leukemia and from healthy donors of the same blood group (by the ABO and Rh systems).*

Differentiation of the Antigenic Properties of the Red Cells of Patients with Leukemia and Healthy Donors of the Same Blood Group by the Specific Delay of Precipitation Test in Agar

Details of tests	Patients with				Total	Healthy donors
	chronic lymphatic leukemia	chronic myeloid leukemia	acute leukemia (hemocytoblastosis)	aleukemia leukemia		
Total number of tests	91	132	36	34	293	167
Number of tests giving a positive result	79	124	35	24	262	0
Percentage of positive tests	87	94	97	70	89	0

The red cells were carefully separated from the leucocytes by preparation of a blood clot (the clot was washed three times with physiological saline and then the pale, upper part was cut away with scissors). The red cells were then suspended 1:5 in physiological saline at pH = 7.2, and homogenized in a Waring-type blender in the cold for 10 minutes. The homogenate was clarified by centrifugation at 10,000 rpm for 60 minutes. The precipitate was discarded. The supernatant fluid was preserved with merthiolate and was used as antigen. The protein content of each antigen in mg was determined. The antigens were stored in the cold. Before the experiment the antigens were diluted so that 1 ml of each antigen contained the same quantity of protein. Red cells of healthy donors of the same group were pooled before preparation of antigen.

In the performance of the specific delay of precipitation test in agar, an excess of antigen from healthy donors' red cells was injected in the first place.

In these conditions the antiserum gave no precipitation lines during the test with healthy donors' red cells. If the dose of antigen introduced into the agar was too small, only a part of the antigenic spectrum of the healthy donors' red cells was suppressed.

Equal doses of all the test antigens were used in the reactions.

EXPERIMENTAL RESULTS

During the performance of the specific precipitation in agar test with antiserum to leukemic splenic tissue and with antigens from the red cells of patients with leukemia and from healthy donors, it was found that the former gave a more complex precipitation spectrum than the latter.

This was shown particularly clearly by the specific delay of precipitation test in agar (Figs. 1-3).

The results of this test are summarized in the table. As may be seen from the table, during the examination of the blood of leukemia patients positive results were obtained in 262 of the 293 tests. The highest percentage of positive results was obtained in the tests on patients with acute leukemia (97), the lowest – but still considerable (70) – in the tests with aleukemic leukemia. The possibility of differentiation between the red cells of patients with aleukemic leukemia and of healthy donors by means of their antigenic properties is undoubtedly of great interest.

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The number of positive tests was related to the precipitation titer of the antiserum used in the reaction and to the spectrum of the antibodies which it contained. In our experiments the greatest activity was shown by the antiserum to tissue of the whole spleen, but the other two leukemic antisera also gave good results. The antserum obtained by immunization of rabbits with the nuclear fraction of leukemic splenic tissue, for instance, gave clearly positive results in the specific delay of precipitation test with antigens from red cells of patients with leukemia, whereas antiserum to the nuclear fraction of normal splenic tissue did not react in the delaying test with either the red cells of healthy donors or the red cells of patients with leukemia.

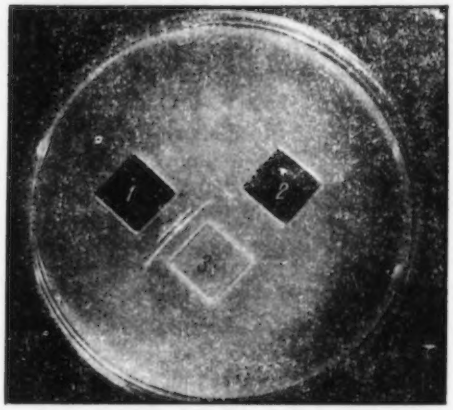

Fig. 1. Specific delay of precipitation test in agar with antiserum to leukemic splenic tissue and antigens from red cells of patients and healthy donors of the same blood group. The agar contained antigens from healthy donors' red cells: hole 1) red cells from patients with chronic lymphatic leukemia; hole 2) healthy donors' red cells; hole 3) antiserum to tissue from a leukemic spleen.

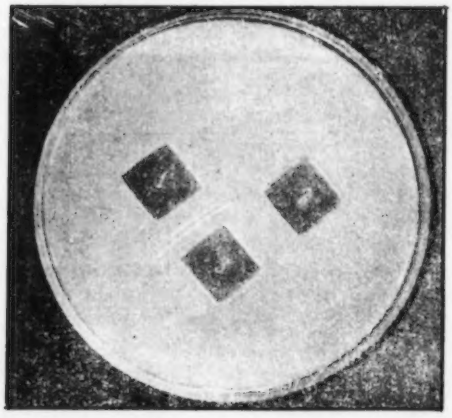

Fig. 2. The same as in Fig. 1. In hole 1) red cells from a patient with chronic lymphatic leukemia; hole 2) healthy donors's red cells; hole 3) antiserum to tissue from a leukemic spleen.

During the performance of the specific delay of precipitation test with antigens from patients with lymphogranulomatosis and with antiserum to tissue from the whole leukemic spleen, no positive results were obtained in any of the 12 tests. The red cells of a patient with the clinical diagnosis of "hypoplastic anemia", on the other hand, gave a clearly marked positive reaction (Fig. 3).

The investigations which we carried out showed that it is possible to differentiate between the red cells of patients with leukemia and those of healthy donors by means of their antigenic properties.

Fig. 3. The same as in Figs. 1 and 2. In hole 1) red cells from a patient with chronic lymphatic leukemia; hole 2) red cells from a patient with hypoplastic anemia; hole 3) red cells from a patient with an exacerbation of chronic myeloid leukemia, hole 4) healthy donors' red cells; the central hole contained antiserum to tissue from a leukemic spleen.

As a result of these investigations, many new problems arise: to what is due the change in the antigenic properties of the red cells in leukemia, what is the character of the changes, do the red cells of patients with leukemia acquire the power of causing the production of antibodies which will react specifically with the altered red cells, and are these changes peculiar to leukemia or do they occur in other neoplastic disease [1]. In subsequent research we shall endeavor to shed light on these problems.

SUMMARY

By their antigenic properties the erythrocytes of leukemic patients differ from those of healthy donors of the same blood group (by the ABO and Rh systems). These differences are revealed in the reaction of specific inhibition of precipitation in agar with the antiserum obtained by immunization of rabbits with the leukemic splenic tissue.

By inhibiting precipitation through introduction of surplus erythrocytes of healthy donors into agar, the antiserum would not reveal their full range (incomplete inhibition) or, in reacting with them, would not form any lines of precipitation at all. It was against this background that the lines of precipitation formed in reaction of the antiserum with the erythrocytic antigens of leukemic patients were especially pronounced.

In 262 tests out of 293 (in 89% of all cases) the erythrocytes of patients suffering from various forms of leukemia could be differentiated from those of healthy donors by their antigenic properties.

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THE MOTOR CHRONAXIE IN PATIENTS WITH MALIGNANT AND BENIGN TUMORS OR WITH OTHER DISEASES

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Having observed changes in the motor chronaxie in experiments on animals during growth of a malignant tumor, and having successfully employed these changes as a diagnostic test [1], we have verified our observations in the clinical field. The chronaxie of the flexors and extensors of the right wrist was measured. In all, 450 persons were examined, 190 of whom were donors. The values obtained for the chronaxie and the rheobase in these donors differed in no way from the physiological standards (Table 1).

TABLE 1

Chronaxie and Rheobase in Donors at the Moscow Regional Blood Transfusion Station (mean values according to age groups)

Group	Flexors (median n.)		Extensors (ulnar and radial nn.)	
	Rheobase	Chronaxie	Rheobase	Chronaxie
I				
From 18 to 25 years	41.6	0.39	34.7	0.47
II				
From 25 to 40 years	40.5	0.34	28.7	0.64
III				
40 years and over	39.9	0.30	30.8	0.54

168 patients were examined, suffering from other diseases, including diseases of the heart, lungs, gastrointestinal tract, skin, endocrine and nervous systems. The mean values of the chronaxie and rheobase in this group of patients, who acted as controls, showed no essential difference from those in the healthy subjects (Table 2).

Attention is drawn to the fact that within this group there were characteristic features. For example, in patients with acute disorders of the cardiac rhythm no clear response to stimulation could be detected, and the readings were extremely fluid, varying from small to large figures and back again. In patients with acute inflammatory conditions, a reduction in the chronaxie was observed, and in patients with chronic inflammatory conditions it was increased. In some cases the value of the subordination index exceeded three.

TABLE 2

The Chronaxie and Rheobase in Patients with Other Diseases (mean results)

Groups of patients with other diseases	Flexors (median n.)		Extensors (ulnar and radial nn.)	
	Rheobase	Chronaxie	Rheobase	Chronaxie
I				
(Diseases of the cardiovascular system)	39.91	0.34	41.28	0.60
II				
(Diseases of the respiratory organs)	45.94	0.31	44.55	0.43
III				
(Diseases of the gastrointestinal tract)	40.61	0.28	38.56	0.71

TABLE 3

The Chronaxie and Rheobase in Patients with Malignant Tumors in Various Situations

Patients' surname	Age, in years	Diagnosis	Duration of disease (in years)	Chronaxie values				Bouguignon's coefficient	Notes
				flexors		extensors			
				rheobase	chronaxie	rheobase	chronaxie		
B-n	35	Recurrence of sarcoma of the soft tissue of the head	2	47,5	0,09	40,0	1,08	+12	The ratio is shown as positive when the chronaxie of the extensors exceeded that of the flexors, and negative when the chronaxie of the flexors exceeded that of the extensors
M-v	51	Carcinoma of the stomach	—	47,5	1,18	50,0	0,33	—3,6	
K-v	53	Carcinoma of the stomach with omentum metastasis	3	55,0	0,80	52,5	0,2	—4	
G-ch	42	Carcinoma of the breast with metastases in the lymphatic glands	—	29,0	0,57	26,5	0,12	—4,6	
A-n	62	Carcinoma of the stomach	1	60,0	0,19	63,0	1,08	+5,6	
V-va	42	Carcinoma of the breast	—	52,5	0,24	24,0	0,89	+3,7	
B-n	42	Carcinoma of the stomach with metastases in the pancreas and omentum	1	60,0	0,09	40,0	0,57	+6,3	
M-r	51	Carcinoma of the breast	—	38,5	0,17	20,0	0,94	+5,5	
K-va	26	Angiosarcoma of the soft tissues of the face	4	63,0	0,14	75,0	0,04	—3,5	
Yu-n	62	Melanoma of the leg	—	76,0	0,08	58,0	0,24	+3	
K-i	26	Stage III lymphogranulomatosis	1	39,5	0,33	33,0	1,03	+3,1	
Sh-va	26	Stage III lymphogranulomatosis	1	17,5	0,14	27,5	0,61	+4,3	
F-v	63	Stage II lymphogranulomatosis	—	49,5	0,14	27,5	0,81	+5,7	
P-kh	16	Stage II lymphogranulomatosis	—	35,5	0,35	27,5	0,61	+1,7	

TABLE 4

The Chronaxie and Rheobase in Patients with Benign Tumors

Patients surname	Age, in years	Diagnosis	Duration of disease (in years)	Chronaxie values				Bourguignon's coefficient
				flexors		extensors		
				rheobase	chronaxie	rheobase	chronaxie	
N-va	32	Hemangioma of left half of face	32	29,5	0,17	29,0	0,28	+1,6
P-v	43	Telangioectatic neurofibromatosis	1	40,0	0,14	35,0	0,38	+2,7
M-va	38	Polyposis of the stomach	15	88,0	0,21	48,0	0,42	+2
V-va	64	Endometritis	—	32,5	0,33	35,0	0,42	+1,2
Zh-va	34	Fibrocystic disease of the breast	2	38,0	0,14	35,5	0,30	+2
K-va	26	Fibroma of the anterior abdominal wall	—	37,0	0,38	27,5	0,66	+1,7
L-aya	43	Fibrocystic disease of the breast	8	25,5	0,32	32,0	0,66	+2
B-va	52	Hemangioma of the skin of face	52	26,5	0,26	31,0	0,52	+2
I-v	53	Hemangio-endothelioma of the left thight	—	35,0	0,80	27,5	1,08	+1,3
K-na	34	Fibrocystic disease of the breast	—	31,5	0,27	28,5	0,28	1

The patients with neoplastic disease formed a group of 92 persons, the main sites of the tumors being the stomach, breast, skin, soft tissues of the limbs and the lymphatic glands. There were 79 patients with malignant tumors and 13 with benign. Among the patients with malignant tumors were some admitted for the first time and as yet untreated, and others readmitted. In the untreated patients the chronaxie and the subordination index were sharply altered, usually being increased; often the relationships between the values of the chronaxie were distorted. The changes in the chronaxie and the subordination index were independent of the site of the tumor (Table 3).

In patients with benign tumors the flexor and extensor chronaxies and their relation to each other were close to normal (Table 4).

In the group of patients with malignant tumors and treated by drugs, by surgery or by radiotherapy, the values of the chronaxie were close to normal; in cases where the widespread nature of the disease precluded any attempt at radical operation or in which drug or radiation treatment was unsuccessful, the chronaxie readings showed quantitative changes which were similar to the pattern of these readings in patients with untreated malignant tumors (Table 5).

Thus the presence of a malignant tumor in the body is accompanied by functional changes in the nervous system, easily recorded at the periphery by the method of chronaximetry, independently of the site of the disease. After the surgical removal of the malignant tumor or after other effective treatment, the chronaxie and the subordination index return to normal.

We discovered changes in the chronaxie, leading to an increase in the subordination index or to its distortion, not only in patients with malignant tumors but also in several patients with other diseases. There is thus no possibility of using the results of chronaximetry as a diagnostic test of cancer, in spite of the fact that these results are of definite interest in assessing the functional state of the nervous system and the general condition of the patient.

SUMMARY

Peculiar quantitative characteristics were noted in the motor chronaxia in experiments on animals with the presence of malignant tumors. Using these data of chronaxia as a diagnostic test, the authors subjected their observations to verification in clinical conditions. Studies were conducted on 450 patients, including those with the presence of a malignant tumor, benign tumor, cases with various internal diseases and the group of healthy donors. It appeared that in patients with malignant tumors there was a shift of the cronaxia values in the direction of their increase and pronounced disturbance of the interrelationship of chronaxia registered from the flexors and

TABLE 5

The Chronaxie and Rheobase in Patients with Benign Tumors

Patient's surname	Age, in years	Diagnosis	Duration of disease (in years)	Chronaxie values				Bourguignon's coefficient
				flexors		extensors		
				rheobase	chronaxie	rheobase	chronaxie	
P-na-N-n	58	Carcinoma of the breast	—	34,0	0,80	39,5	0,56	—1,4
F-e	58	The same	3	55,0	0,35	42,5	0,61	+1,7
S-va	36	The same (with metastases in the lymphatic glands)	—	42,5	0,12	38,0	0,40	+3,3
R-v	76	Carcinoma of the stomach	1	41,5	0,56	35,0	0,56	1
F-v	55	" "	1	31,5	0,56	31,5	0,71	+1,3
K-na	63	Malignant polyp of the stomach	1	35,0	0,35	27,5	0,71	+2
S-v	49	Carcinoma of the esophagus	11	44,0	0,14	31,0	1,83	+13
K-va	45	Carcinoma of the stomach	1	47,5	0,26	48,0	0,47	+1,8
Shch-v	56	The same (with metastases in the omentum)	—	42,5	0,14	34,0	1,13	+8,1
M-va	55	Malignant polyp of the stomach	—	32,5	0,14	37,5	0,28	+2
F-o	27	A typical form of lymphogranulomatosis	—	55,0	0,47	30,0	0,42	—1,1
L-n	22	Stage III lymphogranulomatosis	2	40,0	0,24	30,0	0,14	—1,7
P-v	33	Mediastinal form of lymphogranulomatosis	2	41,0	0,42	32,0	0,47	+1,1
K-v	18	Stage IV lymphogranulomatosis	2	47,5	0,19	45,0	0,19	1
G-na	56	The same	5	48,5	0,24	37,5	Generalized response	
R-v	23	Stage II lymphogranulomatosis	5	47,5	0,18	40,0	0,06	—3
N-v	26	The same	—	39,5	0,75	40,0	0,56	—1,3
Z-na	21	Stage III lymphogranulomatosis	—	30,0	0,19	30,0	3,96	+20,8
D-n	38	The same	—	37,5	0,94	31,5	0,94	1

extensors of the right hand. The changes in chronaxia were registered irrespective of the localization of the tumor. There were no significant quantitative changes in chronaxia in presence of a benign tumor. After removal of or treatment of the malignant tumor the chronaxia normalizes. Similar changes in the chronaxia and in the subordination index were registered both in patients with malignant tumors, and in those with skin diseases (psoriasis) and diseases of the thyroid, gland, stomach and duodenum. Thus, the possibility of employment of chronaxia as criteria for the diagnosis of malignant tumors is rejected. However, it still retains its value as an auxiliary test, when combined with other methods of clinica observation, in evaluation of the general condition of the patient.

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EXPERIMENTAL BIOLOGY

THE ACTION OF CORTISONE ON THE OVARIES OF THE LOACH

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The functional connection between the adrenal cortex and the ovaries has been shown by several experimental investigations carried out on mammals [8, 15]. In the adrenal cortex of sexually mature females cyclic changes are observed which are synchronous with the changes taking place in the ovaries during the sexual cycle. After castration hypertrophy of the adrenal cortex takes place. The adrenal cortical hormones alter the sensitivity of the ovaries to the gonadotropic hormone [11]. Nevertheless the question of the presence of similar functional connections in members of other classes of vertebrates has hardly been investigated at all. Only in the viviparous cartilaginous fishes has an increase in the size of the interrenal gland been described during pregnancy [17].

The physiological mechanism of the transformation of female fishes into the spawning state has received little study. The majority of experimental research has been devoted to the study of only one of the links of this mechanism – the action of the gonadotropic hormones of the hypophysis on the ovaries [1]. It was found that the luteinizing hormone is the "spawning hormone" of the teleost fishes, which stimulates maturation of the oocytes and ovulation [7], i.e. the change from stage IV to stage V of maturity, according to the accepted terminology of ichthyologist [9]. The follicle-stimulating, lactogenic and adrenocorticotropic hormones themselves do not cause this reaction, but they increase the sensitivity of the ovaries to the luteinizing hormone [7]. It was also shown that certain steroid hormones (methyltestosterone, progesterone and desoxycorticosterone) may also cause maturation of oocytes and ovulation in female loach [6], but the mode of action of these hormones is not yet explained.

The hypohysis of the bony fishes contains biologically active substances whose action on mammals and birds is similar to that of the follicle-stimulating [4], luteinizing [4, 19], lactogenic [14], somatotropic [16], thyrotropic and adrenocorticotropic [18] hormones. After intramuscular injection of female loach with a suspension of these substances from the hypophysis of fish, maturation of oocytes and ovulation (at a temperature of 19-20°) usually begin on the average after 24 hours [3]. After injection of luteinizing hormone or chorionic gonadotropin, however, or of the blood or urine of pregnant women, this reaction appears much later, usually 39-48 hours after the injection. The earlier reaction of the female loach to the injection of the substances from the hypophysis of fish, compared with the reaction to the purified luteinizing hormone, may depend on the fact that the hypophysis stimulates not only the function of the ovaries but also the activity of the other endocrine glands, hormones from which act synergistically with the luteinizing hormone on the ovaries. The adrenocorticotropic hormone in particular, evidently stimulates the activity of the interrenal gland, which secretes some form of glycocorticoid into the blood stream. It was shown that this glycocorticoid is present in considerable quantity in the blood plasma of fishes during the spawning period [12].

It was therefore decided to study the action of cortisone on the ovaries of bony fishes and to investigate its action on the sensitivity of the ovaries to luteinizing hormone, since this would enable the part played by glycocorticoids in the transformation of female fishes into the spawning state to be established.

EXPERIMENTAL METHOD

Experiments were carried out on adult female loach (Misgurnus fossilis), caught in January 1958 in the neighborhood of the town of Stanislav. In winter the ovaries of the female loach are in stage IV of maturity, i.e. they contain large, immature oocytes, which are completing their period of growth and deposition of lutein. Their transformation to stage V of maturity, i.e. the appearance of mature oocytes and of ovulation is never accomplished under laboratory conditions spontaneously, but may easily be brought about by injection of material from the hypophysis of fish [3, 10] or mammals [5], and also by human tissue and tissue fluids containing chorionic gonadotropin [5]. From 40 to 48 hours after the intramuscular injection of a preparation of luteinizing hormone, or of a suspension or extract of tissues containing this hormone or chorionic gonadotropin, the female loach begins to lay mature spawn in the water. If the mature females are taken in the hand or gently massaged with the finger along the abdomen from head to tail, the spawn flows freely from the reproductive orifice of the fish. Without injection of gonadotropic hormones or, if the dose of hormone injected is below threshold, the ovaries remain in stage IV of maturity and no spawn emerges in response to abdominal massage.

Loach were injected with the following hormone preparations:

1) Cortisone acetate, manufactured by Biddle, Sawyer and Co. (London). 1 ml of an aqueous suspension of this preparation contained 25 mg of cortisone acetate. Before injection, the suspension was diluted with distilled water.

2) A purified preparation of luteinizing hormone from pigs' hypophyses, produced in the experimental manufacturing laboratory of the All-Union Institute of Experimental Endocrinology (1 unit of activity of the hormone was contained in 3 mg of the dry powder). The preparation was dissolved in distilled water immediately before injection.

Before the experiments the loaches were kept in a large tank with frequently changed tap water. During the experiments they were kept in glass tanks containing water but no sand or plants, at a temperature of 18-20°, and were given no food. The loaches were examined 24, 48 and 72 hours after the injection, and in cases when it was necessary to know exactly the time of onset of the reaction, they were examined every 1-2 hours for certain periods of time. If 72 hours after the injection no spawn had been expelled from the genital aperture, the fish was opened up so that the condition of its ovaries could be determined. In several experiments in which cortisone acetate was injected without other hormones, the fishes were opened up 48 hours after the injection.

Results of the Simultaneous Action of Cortisone and Luteinizing Hormone on Female Loach

Series of experiments	Dose of cortisone acetate, in mg	Dose of luteinizing hormone, in mg	Number of female loach	Number of females		
				laying mature ova	in which maturation of oocytes only took place	in which ovulation of immature follicles alone took place
1	1	–	5	0	5	0
2	1	1	5	5	0	0
3	1	0.5	5	5	0	0
4	1	0.2	5	2	2	0
5	0.5	–	5	0	3	0
6	0.5	0.5	5	3	2	0
7	0.5	0.2	5	0	5	0
8	–	1	6	0	0	1
9	–	0.5	5	0	0	0
10	–	0.2	6	0	0	1

EXPERIMENTAL RESULTS

After a single intramuscular injection of 1-5 mg of cortisone acetate into the female loaches, maturation of all or almost all the large oocytes in their ovaries took place. Thanks to the fusion of the droplets of lutein into a homogeneous, translucent mass, these oocytes themselves became translucent. No ovulation developed, however. After a few days the mature oocytes which remained inside their follicles showed degenerative changes and underwent resorption.

If these mature oocytes were artificially extracted and fertilized with sperm obtained by compressing the testes of the loach between two watch glasses, they developed normally. In one large female loach, opened up 48 hours after injection of 7 mg of cortisone acetate, several hundred mature ova were artificially extracted and fertilized. 77.9% of these ova developed normally, and from them larvae were hatched, after 3-4 days, which survived in the laboratory without food for about two weeks.

After injection of 0.5 mg of cortisone acetate, maturation of almost all the large oocytes took place in only a few female loach. In the remainder, the ovaries stayed in stage IV of maturity, although among the mass of immature oocytes inside the follicles, here and there translucent mature oocytes were encountered, singly or in groups.

In order to discover the result of the simultaneous action of cortisone and luteinizing hormone, female loaches weighing from 35 to 45 g were given injections of 0.5-1.0 mg of cortisone acetate into the muscles of the left side of the body, and 5-10 minutes later they were injected, in the muscles of the right side of the body, with various subthreshold doses of luteinizing hormone. Control females of the same weight were given injections of cortisone acetate alone or subthreshold doses of luteinizing hormone without cortisone. The results of these experiments are shown in the table.

As may be seen from the table, after the injection of subthreshold doses of luteinizing hormone soon after cortisone acetate, a valid transformation of the ovaries of the female loach from stage IV to stage V of maturity took place, leading to the excretion of mature spawn. The smaller the dose of cortisone acetate injected, the more luteinizing hormone was it necessary to give in order to obtain a valid reaction. In their action on the ovaries of the loach, cortisone and luteinizing hormone were synergists.

Furthermore, if the female loaches were injected with 1 mg of cortisone acetate and 1 mg of luteinizing hormone, they began to excrete mature ova only 22-28 hours after injection, i.e. at the same time as after injection of the hypophyseal substances of the loach, and 12-18 hours earlier than after injection of even large doses of purified luteinizing hormone without cortisone.

For an explanation of the mechanism of action of cortisone on the ovaries of the loach, we carried out experiments to study the action of this hormone on pieces of the ovaries in vitro.

B. N. Kazanskii [2] showed that if from female sturgeon or salmon, in the last 2-3 hours befores maturation, pieces of ovary are removed and removed in ovarian fluid, maturation of the oocytes and ovulation are effected in vitro. He postulated that ovulation and maturation of ova outside the body are possible from the time when morphologically visible changes have taken place in the relationships between the oocytes and the cells of the follicular epithelium.

We have repeatedly observed that if, a short time after injection of a suspension of hypophysis, luteinizing hormone, chorionic gonadotropin or the urine of pregnant women, the female loaches are opened up and pieces of their ovaries are placed in Ringer's or Holtfeter's solution [13], then normal maturation of the oocytes and ovulation take place in these pieces at approximately the same times as in the female body (sometimes even after existence outside the body for many hours). However, our numerous attempts to achieve maturation of oocytes and ovulation in pieces of ovary from the loach by the action on them of hypophyseal substances from the loach or of preparations of gonadotropic hormones, added to them in vitro, have never yielded positive results. Only when the suspension of hypophyseal substances of gonadotropic hormone was injected into the body of the fish, and the pieces of ovary were excised and placed in Ringer's solution not sooner than 3 hours after injection, did maturation of the oocytes take place in them; if they were excised after 12 hours or more, ovulation also took place. Evidently the gonadotropic hormone must act on the ovaries for a definite period of time within the body of the fish, and only at the conclusion of this time can the processes which it has stimulated be continued outside the body – in isolated pieces of ovary placed in physiological saline.

The results of the experiments with cortisone were interesting. Small pieces of the ovaries, excised from female loaches in stage IV of maturity, were placed in porcelain dishes with 20 ml of Ringer's solution. To this solution in the different dishes were added 1 mg of cortisone acetate, a suspension of the hypophyseal substance of one or two loaches or 1-3 mg of luteinizing hormone. The hypophyseal suspension and the luteinizing hormone caused no visible changes in the pieces of ovary. After the addition of cortisone acetate to the Ringer's solution, in every case after various times, from 26 to 32 hours, the greater part of the large oocytes had matured and become translucent. Hence cortisone acts directly on the ovaries and causes maturation of oocytes of the loach in vitro in the absence of any other hormone.

The addition to the Ringer's solution of a suspension of the substance of one or two hypophyses of the loaches at the same time as the cortisone acetate did not lead to the development of ovulation in the pieces of ovary in vitro. Maturation of the oocytes in these pieces took place at the same times as after the action of cortisone acetate alone. Addition of the hypophyseal suspension to the solution at various intervals of time after the cortisone acetate, whether before or after the onset of maturation of the oocytes in vitro, also did not cause ovulation.

The experimental findings suggest that some glycocorticoid, close in its properties to cortisone, takes part in the physiological mechanism of transformation of female bony fishes from stage IV to stage V of maturity. This hormone probably has an effect on the metabolism of the fish during its transformation to the spawning state, and at the same time acts directly on the ovaries as a synergist of the luteinizing hormone.

SUMMARY

Injection of cortisone to loaches (Misgurnus fossilis) caused maturation of oocytes without ovulation. The same could be observed in in vitro experiments. With the administration of cortisone and subliminal doses of purified luteinizing hormone the ovulation and oviposition of mature ova occurred 12 to 18 hours earlier than in the instances of large doses of luteinizing hormone without cortisone.

Evidently a certain glucocorticoid, similar to cortisone, takes part in the physiological mechanism causing the fish to change into conditions of spawning. It acts directly on the ovaries and is a synergyst of the luteinizing hormone.

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CHANGES IN THE SORPTIVE PROPERTIES OF CERTAIN TISSUES OF THE RAT DURING AGING

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According to the research carried out at Khar'kov University with aging a gradual extinction of the processes of self-renewal takes place [6, 7]. Since protein is the main component of the protoplasm and the material basis of all vital processes, it is natural to connect these changes in metabolism with qualitative and quantitative changes taking place in the protein substrate.

Ruzicka [15-17] considered that during aging there is primarily a physicochemical alteration in the protein: there is a fall in its electric charge and in its degree of ionization, in consequence of which there develops dehydration, thickening of colloids, replacement of more labile by stable compounds, increased viscosity and a reduction in the degree of dispersion. This complex group of changes as a whole was called by Ruzicka protoplasmic hysteresis, and on it was based his colloid-chemical theory of aging.

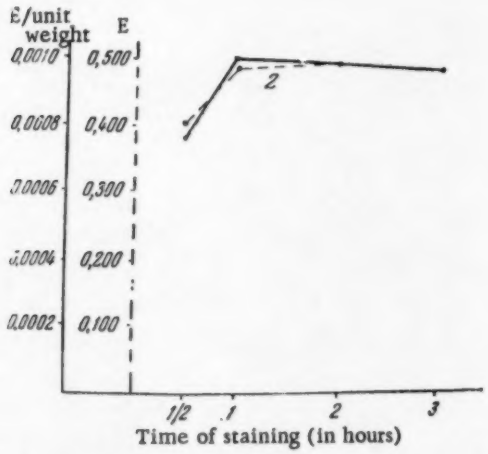

Fig. 1. Sorption of phenol red by muscle (1) and corneal (2) tissue of rats after staining for different lengths of time. E – extinction

Recently, in work by I. N. Bulankin and M. A. Blyumina [3], the hypothesis has been put forward of an "age increase in the cyclic structures in the protein molecule or an age stabilization of the cyclic structures already present in the protein molecule."

We thought it interesting to consider the problem of the qualitative changes occurring in proteins during the process of aging, on the basis of the denaturation theory of excitation and injury, put forward by D. N. Nasonov and V. Ya. Aleksandrov [8].

It was shown by D. N. Nasonov and his co-workers, in a series of investigations, that under the influence of various stimuli – i.e. in response to excitation and injury – the protoplasm begins to adsorb vital stains more intensively [5, 8, 9, 11, 12].

The increased sorption of acid and basic stains is associated with denaturation changes in the protein, and results from the appearance of new, ionized groups in the protein molecules. Possibly, during aging, the tissues cells reach a state which, in its nature, closely resembles that which arises in response to the action of stimuli, so that similar changes (of the denaturation type) must take place then in the protoplasm, and in particular an increased sorption of stains would be expected.

The present work was devoted to an experimental verification of this hypothesis. We also thought that it would be interesting to find out whether the blood proteins are also affected in a similar manner.

EXPERIMENTAL METHOD

The investigation was carried out on white rats which had been reared in the laboratory. Tissues from rats of four ages were investigated: 3, 6, 18 and 24 months.

The experimental material consisted of the muscles (soleus and extensor digitorum longus), the cerebral hemispheres, the cornea and the blood serum. The animals were killed by decapitation. Blood was collected in tubes, in which the serum was allowed to separate.

TABLE 1

Sorption of Vital Stains by Muscle Tissue of Rats of Different Ages (in Extinctions per mg Dry Tissue) (the arithmetic mean ± mean square deviation is given)

Object and dye	Age, in months			
	3	6	18	24
Soleus muscle (phenol red, 0.06%)	0.0040 ±0.00014	0.0052 ±0.00011	0.0062 ±0.00037	0.0081 ±0.00032
Number of experiments	14	24	13	8
Extensor digitorum longus muscle (neutral red, 0.05%)	0.0033 ±0.00012	0.0042 ±0.00017	0.0066 ±0.00034	0.0054 ±0.00025
Number of experiments	11	15	9	11

TABLE 2

Sorption of Pheno Red (0.05%) by Brain and Corneal Tissues of Rats of Different Ages (in extinctions)

Object	Age, in months			
	3	6	18	24
Brain tissue	0.234 ±0.013	0.318 ±0.015	0.368 ±0.016	0.460 ±0.025
Number of experiments	13	15	11	10
Corneal tissue	0.306 ±0.009	0.315 ±0.008	0.353 ±0.021	0.411 ±0.016
Number of experiments	14	16	10	11

TABLE 3

Sorption of Methylene Blue (9 mg%) by the Serum Proteins of Rats of Different Ages (quantity of dye in mg/100 mg of albumin)

Object	Age, in months			
	3	6	18	24
Blood serum	273.7 ±18.2	410.7 ±26.8	511.5 ±38.9	533.1 ±12.1
Number of experiments	10	11	8	8

The organs for testing were placed in Ringer's solution (muscles and brain for 5 minutes, cornea for 10 minutes).

In the investigation of the sorptive properties of the muscles, brain and cornea a quantitative method of vital staining was used. The soleus muscle, brain and cornea were stained with an acid stain – phenol red (the concentration used for the brain and cornea was 0.05%, and for the muscle 0.06%), and the extensor digitorum longus muscle was stained with a basic stain – neutral red (0.05%). In staining all the tissues care was taken as far as possible to avoid injury.

For staining the muscles the solutions were aerated; for staining the brain the lay of stain above it did not exceed 2 mm in thickness. After extraction of the tissues from the stain, no perceptible abnormalities were found under the microscope.

The criterion of a good condition of the muscle which was used was only little changed in its excitation throughout the experiment. Its fall did not exceed 20%.

The soleus muscle was stained in an incubator at 35° for one hour, i.e. until the establishment of equilibrium in the distribution of the acid stain between the muscle and the solution (Fig. 1). The extensor digitorum longus muscle was stained in the same way for 5 minutes.

After they had been removed from the stain the muscles were rinsed, dried on filter paper and extracted with 70% alcohol acidified with 7% sulfuric acid in the case of the acid stain and acidified with 2% sulfuric acid in the case of the basic stain. In both cases the extracted dyes were estimated photometrically on the photoelectric colorimeter, after which the muscles were dried to constant weight and the sorption of the stain was calculated in relative units (extinction per mg dry tissue).

The cornea was stained in toto for 40 minutes at 37° (see Fig. 1), after which the tissue was washed, and pieces were cut from it with a circular punch (d = 4.5 mm), which were placed in acidified alcohol in order to extract the stain.

The brain was stained in the same way, washed in Ringer's solution, and pieces were cut from the parietal and temporal areas of the cerebral hemispheres, by means of a circular punch (d = 8 mm), and used for extraction of the stain.

The extracts from the brain and cornea were estimated photometrically in small dishes with a Pulfrich's photometer. The quantities of stain adsorbed were compared as "extinctions" shown by the readings of the photometer.

For the investigation of the sorptive properties of the serum proteins, the method used was that of diffusion of free dye in a gelatin gel, as devised by A. D. Braun [2] and slightly modified by ourselves.

The serum protein concentration was determined by the biuret method, and the albumin-globulin ratio nephelometrically [1]. The serum was stained by a basic dye – methylene blue – in an initial concentration of 9 mg%. Fixation of dye was calculated as mg/ 100 mg of albumin, since of the serum proteins, it is only the albumins which fix the dye [13].

The experiments were carried out over a period of 3 years, at different seasons. Control experiments were performed at the same time on 6-month old animals. The sorptive power of the tissues of the 6-month old rats was practically unchanged.

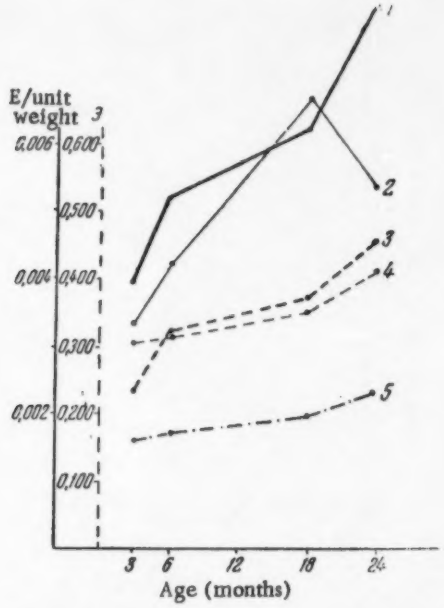

Fig. 2. Changes in the sorptive properties of muscle tissue (soleus, stained with phenol red – 1; extensor digitorum longus, stained with neutral red – 2), brain (3) and corneal (4) tissue of rats of different ages. Changes in the pigment content of the brain tissue during aging (5). E – extinction.

EXPERIMENTAL RESULTS

As can be seen from the data in Tables 1-3, an increase in the sorptive power of the tissue and serum proteins took place during aging. The sharpest increase in sorption of dye was observed in the tissues of the brain, muscles and cornea at the age of 18-24 months.

If the sorptive power of the tissue of the 3-month old animals was taken as 100%, then the sorption of dye by tissues of the 2-year old animals was increased in the brain by 96.6%, in the cornea by 34.3%, in the muscle for phenol red by 102.5% and for neutral red by 63.6%.

Attention must be drawn to a peculiarity in the change in sorption of basic dye, consisting of the fact that the sorptive power of the muscle tissue toward neutral red rose comparatively abruptly in the period from 3 to 18 months, and then fell considerably at 24 months.

The brain tissue stained extremely weakly, so that a considerable proportion of the extinction during photometry was due to pigment, especially in the younger animals. With increasing age the pigment content of the brain tissue rose (Fig. 2), although the curve of pigment content rose less abruptly than the curve of sorption of dye.

Table 3 illustrates the changes in the sorptive properties of the serum proteins during aging. The character of these changes was the same as that in the tissue proteins of the brain, cornea and muscles, for the sorptive power of the serum proteins of the older animals 2 years) was 94.8% greater than that of the younger animals (3 months)

(Fig. 3). The sharpest changes took place at the age of 3-6 months; the sorptive properties of the serum proteins of the animals aged $1\frac{1}{2}$ and 2 years were practically indistinguishable from each other.

The increase in sorption of dye by the living tissues of the rats with advancing age was evidence of definitive and substantial changes in the proteins on aging.

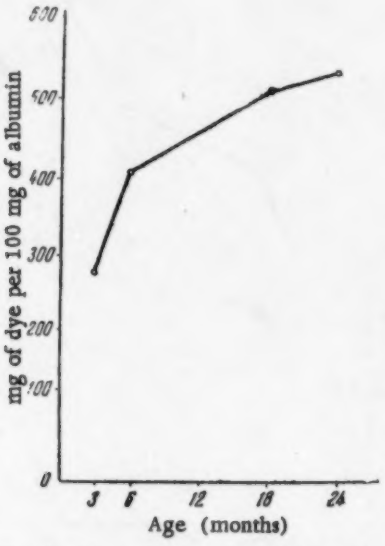

Fig. 3. Changes in the sorptive properties of the serum proteins of rats during aging.

There are several references in the literature to changes in the physicochemical and biochemical properties of proteins during aging, supporting the views of Ruzicka.

Bechhold [14], for instance, describes an increase in the viscosity of the protoplasm of cells during aging. Similar findings were reported by V. N. Nikitin, I. A. Babich and V. S. Lyubavina [10], during an investigation of the viscosity of the serum of cattle. According to the findings of A. V. Nagornyi [6], Hajek [18] and A. O. Voinar [4], the pH of the protoplasm moves in the direction of acid during aging.

If we compare the data in the literature with our experimental findings, it appears possible to postulate that during aging of the body denaturation of the tissue proteins takes place.

SUMMARY

The changes in the sorptive properties of the muscular, nervous, epithelial and connective tissues of white rats were studied in old animals. The quantitative method of vital staining was used for evaluation of the sorptive properties of these tissues. Both basic and acid stains were used.

It was established and statistically confirmed that the sorptive properties of the muscles, brain, cornea and proteins of the blood serum are increased with age, which points to the qualitative changes in the proteins of various tissues. A suggestion is made of the denaturating origin of these changes.

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THE CONDITIONS FOR AND THE HISTOCHEMICAL CHARACTERISTICS OF OSTEOGENIC ACTIVITY OF TRANSITIONAL EPITHELIUM IN GRAFTS

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The morphological changes arising during bone formation as a result of the interaction between transitional epithelium and connective tissue during homografting in guinea pigs have been described in previous commnications [1, 2].

It has been shown that induction is brought about by atypical growth of the transitional epithelium and in several other processes, as a result of which the contents of the cytoplasm of the epithelial cells at a definite level of differentiation are secreted into the underlying connective tissue.

This is particularly the case with a substance of polysaccharide nature, which passes from the epithelium to the underlying tissue under the conditions of transplantation [3, 4].

It is also known that induction of bone tissue is brought about by high alkaline phospatase activity in the transitional epithelium [5, 6], which certain authors [6] are inclined to associate with the mechanism of induction, bearing in mind the important role of this enzyme in osteogenesis.

In view of these findings, it appears desirable to compare the distribution of polysaccharides and alkaline phosphatase in the transitional epithelium when osteogenetic induction is present, or when it is absent.

EXPERIMENTAL METHOD

Three series of experiments were carried out:

I. Homotransplantation of finely cut mucous membrane of the urinary bladder of guinea pigs into the anterior abdominal wall by means of a grooved probe (i.e. with trauma to the connective tissue).

II. The same transplantation, but using fragments suspended in physiological saline, implanted with a syringe (i.e. with minimal trauma to the connective tissue).

III. Homotransplantation of finely cut mucous membrane of the urinary bladder of rabbits into the anterior abdominal wall by means of a grooved probe.

On the 7th-15th day the grafts were fixed by the Shabadash method and also with acetone. As well as the ordinary histological methods which were used in the previous research [1, 2], the Gomori test for alkaline phosphatase and for calcium phosphate, the Koos test for calcium salts and the Schiff periodic acid test for polysaccharides were performed.

EXPERIMENTAL RESULTS

As a result of the proliferation of the transitional epithelium in the transplantates in the guinea pigs on the 7th-10th day, cysts of a dual nature were formed. During the growth of the epithelium along early sclerosing or adult connective tissue cysts developed with a normal, fully differentiated transitional epithelium and with the formation of a basal membrane.

On the 12th-15th day the epithelial lining acquired the structure of ordinary transitional epithelium, consisting of basal, intermediate and large integumentary cells. Such cysts always appeared in the course of transplatation with the syringe, and in some areas of the grafts made with the grooved probe. Induction of bone tissue never occured around these cysts.

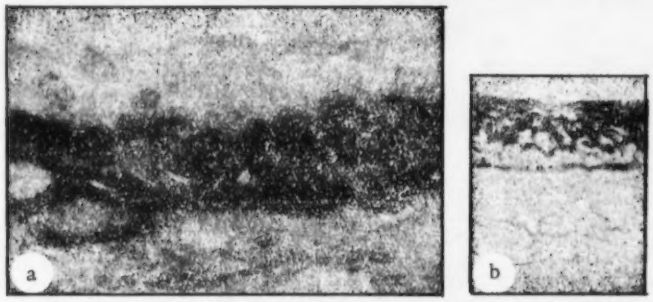

Fig. 1. Normal differentiation of the transitional epithelium in the transplantate; 12 days. Guinea pig.
a)Distribution of alkaline phosphatase. Acetone. Gomori. Objective 40 ×. b)Distribution of glycogen. Shabadash – Schiff periodic acid. Objective 20×.

The second type of cyst was characteristic only of those transplantates made by means of a grooved probe, and it appeared during growth of the epithelial layer over the reactive, young connective tissue. Under these circumstances the transitional epithelium remained incompletely differentiated, integumentary cells were not formed in the epithelial layer, no basal membrane was developed and atypical infiltration of the epithelium into the underlying connective tissue was observed. At the same time osteogenesis was induced there [1, 2].

The distribution of alkaline phosphatase and glycogen in the wall of the cysts with completely differentiated transitional epithelium was very characteristic. A strongly positive reaction for phosphatase was given by the basal layer of epithelial cells, the layer of intermediate cells contained less phosphatase or did not react at all, and the superficial cells were always without phosphatase (Fig. 1, a).

Glycogen showed the opposite gradient in its distribution. In the basal cells it could be found only in the form of isolated, separate droplets, or not at all; in the intermediate cells the quantity of glycogen increased sharply, and during the transition to the surface of the layer the entire cytoplasm of the epithelial cells became filled with polysaccharide in the form of droplets of glycogen and of a homogeneous substance which was not fermented by amylase (Fig. 1, b). Under these circumstances it was evident that glycogen was used up in the formation of this mucin-like substance.

The cysts of transitional epithelium with normal differentiation were surrounded by connective tissue consisting of fibroblastic cells, which gave a positive reaction for phosphatase until the 6th-7th day; on the 10th-17th day a reaction for phosphatase was given by only a narrow border of connective tissue directly in contact with the epithelial lining of the cyst; all the remaining connective tissue around the cyst gave a negative reaction.

Where differentiation was incomplete and atypical areas of infiltration were present, the transitional epithelium had different histochemical features. The usual distribution gradients of alkaline phosphatase and glycogen in the epithelial layer were disturbed; the basal cells of the epithelium, including those invading the depths of the connective tissue, contained not only phosphatase but also a large amount of glycogen, which was concentrated mainly in the most basal areas of their cytoplasm.

The cells thus corresponded to the cells of the intermediate layer and the phosphatase activity in them was less than in the basal cells of the transitional epithelium with normal differentiation.

After the 10th day this epithelium began to secrete into the underlying connective tissue substances from its cytoplasm, including glycogen. This resulted from destruction of the epithelial cells buried in the connective tissue, expulsion of fragments of the cytoplasm of the basal cells (Fig. 2, a), and also from a peculiar form of secretory process, directed both into the depths of the connective tissue and through the surface of the epithelial layer into the cavity of the cysts.

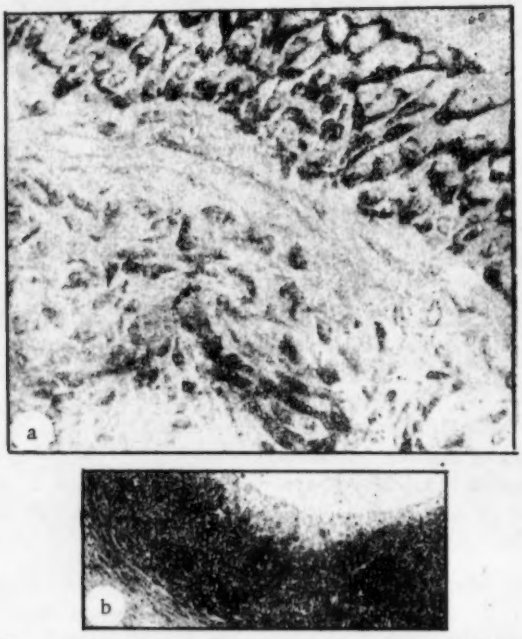

Fig. 2. Epithelium above a focus of induction of osteogenesis. Transplantate; 11 days. Guinea pig.
a) Distribution of glycogen. Shabadash – Schiff periodic acid with counterstaining with hematoxylin. Objective 40 x b) Distribution of alkaline phosphatase. Acetone. Objective 20 x.

The details of these secretory processes in transplantates in guinea pigs have been described previously [1-5]. As a result, after the 10th day osteogenic tissue appeared and bone formation began in the connective tissue underlying the inducing areas of epithelium.

Although the connective tissue surrounding the whole of the transplantate gave a negative reaction for phosphatase on the 10th-12th day and appeared to be adult, a wide zone of tissue lying next to these cysts showed a strongly positive reaction for alkaline phosphatase, characteristic of osteogenic tissue (Fig. 2, b). This reaction was more intensive than in the transitional epithelium situated over it and responsible for the induction.

The formed bone tissue lying in the center of the rudimentary bone gave an intensive Schiff periodic acid reaction and a negative reaction for alkaline phosphatase, and contained calcium salts in the form of confluent granules; droplets of glycogen were contained in the cytoplasm of the immured osteoblasts and in the preosteoblasts.

The osteoblasts, fibers and basic substance of the osteogenic tissue at the periphery of the rudimentary bone gave an intensive reaction for alkaline phosphatase, but contained no calcium salts nor glycogen. Layers of osteoid, giving a weak Schiff reaction, appeared between the cells.

Transplantation in rabbits in all cases gave negative results – no induction of bone tissue was obtained.

In the cysts which arose in the transplantates, complete differentiation of the epithelium with the formation of large superficial cells was observed. Both at the moment of completion of differentiation and in the period of growth of the epithelial layers, they were clearly delineated from the underlying connective tissue; no signs of invasive growth of the epithelium were observed in rabbits, and the differentiated layers had a clear basal membrane. Glycogen was present only in the cells of the intermediate layer and the integumentary cells, and droplets of glycogen could be secreted into the cavity of the cysts from the superficial cells of the epithelium.

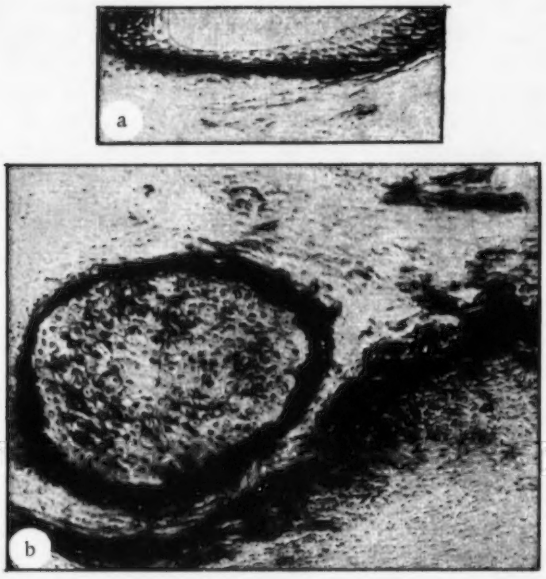

Fig. 3. Transplantate in a rabbit 15 days after grafting. Distribution of alkaline phosphatase. a) In the cyst wall; b) around the transplantate (viewed from above). Acetone. Gomori. Objective 20 ×.

In addition to glycogen, the cytoplasm of the superficial cells and the cysts themselves in the rabbits, as in the guinea pigs, contained polysaccharides in the form of pale pink drops and flocculi which were not fermented by amylase. In some cysts the majority of the superficial cells also contained polysaccharides, probably of mucous origin, in homogeneous form in the superficial areas of their cytoplasm. Polysaccharides of the glycogen type were evidently utilized in the formation of these substances.

Attention was drawn to the large number of capillaries in the cyst walls in rabbits and to their intimate contact with the epithelium, just as occurs in the normal transitional epithelium of the urinary bladder.

Phosphatase was detected in the epithelium only in the basal layer of cells. In the underlying connective tissue a positive reaction for phosphatase was present in a narrow zone (Fig. 3, a) lying immediately beneath the epithelium and containing a large number of collagenous fibers, but not rich in cells, and moreover the reaction for phosphatase was more intensive than in the basal layer of epithelium situated above it. In the tunica propia of the transplantates in the rabbits a feebly positive reaction for phosphatase was often preserved. Furthermore, around the whole of the transplantate there was usually a narrow ring in which alkaline phosphatase activity was observed (Fig. 3, b). On the 15th day this outer ring disappeared.

Thus although the transitional epithelium contained an osteogenic factor, it was evidently able to display its activity only if differentiation of the epithelium had taken place to such an extent that this factor was secreted into the underlying connective tissue. For this to be so it was necessary for the epithelium and connective tissue to be correlated in a particular manner, differing from normal and expressed morphologically by the reactive

state of the connective tissue, the incomplete differentiation of the epithelial layer and the atypical infiltration of the epithelium into the connective tissue.

The trauma of transplantation was sufficient to create these relationships in guinea pigs, in which induction of osteogenesis took place; in rabbits these reactive changes were feebly shown and did not interfere with the normal differentiation of the epithelium – osteogenesis did not arise in the transplantates, as was the case in guinea pigs after implantation through a syringe (with minimal trauma). At the same time growth of the transplanted epithelium took place in both cases.

The histochemical characteristics of normally differentiated transitional epithelium and of epithelium bringing about osteogenic induction were also sufficiently well defined. During induction of osteogenesis, in intimate contact with the connective tissue was a layer of epithelial cells whose cytoplasm contained a large amount of glycogen which could be seen to be secreted from the epithelium into the underlying connective tissue where osteogenesis was being induced. Where induction was absent, as in the normal urinary bladder, the basal cells of the epithelium were without glycogen. During induction of osteogenesis, the epithelial cells next to the connective tissue gave a less intensive reaction for phosphatase than the basal cells of normally differentiated transitional epithelium. Taken as a whole, the inducing cells corresponded in their characteristics to the intermediate cells of the transitional epithelium. Glycogen formation in the layer of transitional epithelium was evidently carried out through hexose monophosphate by the action of phosphorylase, which was found to be present in transitional epithelium [9].

In those transitional epithelial cells in which the alkaline phosphatase activity was high, this glycogen synthesis must have been inhibited on account of dephosphorylation of the Cori ester. In this way the absence of glycogen from the basal cells of the transitional epithelium and its presence in the cells of the overlying layers were presumably explained. The importance of glycogen in the transitional epithelium and the manner of its utilization are unknown [8]. It is obvious in any case that during osteogenic induction, when the phosphatase activity in the layer of transitional epithelium was lowered, a more intensive formation of glycogen in this structure was possible, and this was in fact observed. In certain conditions, this substance, with the histochemical signs of glycogen, obtained access to the underlying connective tissue, where it induced osteogenesis.

The observation was thus confirmed that induction was associated with changes in the gradient of metabolic processes in the epithelial layer, with secretion of intermediate products of polysaccharide conversion into the connective tissue. These active substances were also present in the transitional epithelium of the rabbit, as shown by bone formation in the renal pelvis after ligation of the vessels in experiments in which pelvin was drained away through the ureters [4]. During the conditions of transplantation in the rabbit, however, they were not secreted into the connective tissue.

It must also be emphasized that the same alkaline phosphatase activity was shown in the connective tissue underlying the transitional epithelium in the transplantates as in the tunica propria of the urinary tract [7].

This was the case during both the presence and absence of osteogenesis. In these circumstances, in the transplantates in the rabbit just as in the tunica propria of the normal urinary bladder, a higher phosphatase activity was observed in the underlying connective tissue than in the epithelium itself, and the inducing cells of the epithelium in guinea pigs were characterized by a relatively low phosphatase activity.

These findings were contrary to the view that the phosphatase of the transitional epithelium plays a leading role in its osteogenic activity.

SUMMARY

Comparative histological and histochemical (alkaline phosphatase, polysaccharides) study of transitional epithelium transplants was conducted in guinea pigs and rabbits. It was demonstrated that the osteogenetic effect of transitional epithelium on the connective tissue is associated with the penetration into it of the polysaccharide metabolism intermediate products from the epithelium. High activity of alkaline phosphatase is revealed in the subepithelial connective tissue independent of the presence or absence of osteogenesis. A definite level of epithelial differentiation is necessary for the secretion of the osteogenetic factors into the underlying tissue, this level depending on the condition of connective tissue around the transplant.

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*See English translation.

METHODS

THE TECHNIQUE OF THE ONCOLYSIS REACTION

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In 1910, Freund and Kaminer [2] found that the serum of healthy human subjects and animals possesses the power of dissolving tumor cells in vitro, whereas the serum of animals and human patients suffering from cancer has lost this property.

Attempts have been made to use this phenomenon of oncolysis or cancerolysis, for diagnostic purposes. It was found, however, that loss of the oncolytic power of the sera was not specific for cancer. This must not, however, be regarded as an obstable to the use of the oncolysis reaction in oncological practice. In particular it is of interest to study the changes in the oncolytic activity of serum as a result of the action of antitumor drugs. If in the course of treatment by these drugs, the oncolytic activity of the sera of cancer patients was restored, then this could be used as an indication of the effectiveness of the treatment.

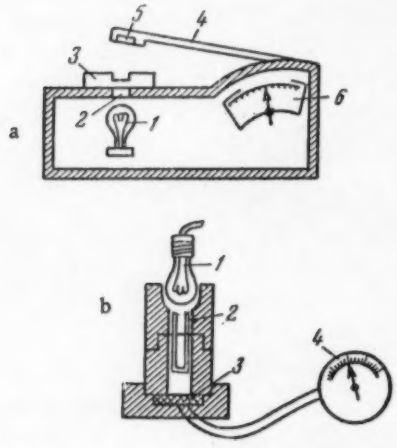

Fig. 1. An outline of the construction of the densitometers. a) Densitometer EEL; b) densitometer as constructed by the author.

The wide use of the oncolysis reaction is also prevented by defects in the method of performance of the test which has so far been most frequently adopted, based on counting the visibly unchanged cells before and after contact with the serum [2]. The first experiments which we performed by this method did not give comparable results. Attempts to develop a more accurate and less laborious technique have not yet been successful.

The aim of the present work was to develop a method of performance of the oncolysis reaction which would give standard results and which would, at the same time, be less laborious than the method of counting the tumor cells.

We decided to use the principle of photometry, starting from the following assumption. If, as a result of the oncolytic activity of the serum, the number of tumor cells in the given medium is reduced, the optical density of the medium must also be reduced correspondingly. The change in the intensity of a beam of light after passing through such a medium, which reflects the degree of lysis of the tumor cells, is easily measured.

For the experiments we used the "Densitometer EEL" (Fig. 1, a). Over the aperture 2, situated directly above the light source 1, is placed the vessel 3 containing the medium whose optical density it is required to measure. On

the vessel rests a handle 4, with a photoelectric cell 5, connected to a galvanometer 6; the scale of the latter is graduated in units of density. The vessel used in measuring the optical density of the medium was a specially made plexiglas plate in which were flat-bottomed hollows measuring 6 mm in diameter and 6.5 mm in depth. In addition to this, we constructed an apparatus whose action was based on the same principle (Fig. 1, b). On to a base is fixed a screw-type lampholder with a small aperture in the center. At the base of the lampholder is mounted a photoelectric cell 3. Above the aperture is placed the light source 1 (the illuminator of an OI-7 microscope). Inside the lampholder is placed a flat-bottomed tube 2, 2.5 mm in diameter, with the medium for testing. The photoelectric cell is connected to a mirror galvanometer 4. A beam of light, passing from above through the tube containing the fluid for testing, and thereby changing in intensity, falls on the photoelectric cell connected to the galvanometer. The galvanometer readings are used for calculation of the oncolytic index.

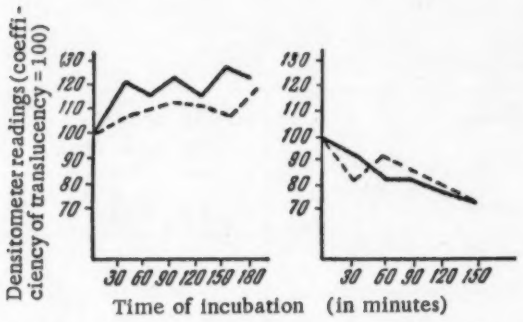

Fig. 2. Typical curves of the optical density of a suspension of tumor cells during incubation at 37°.

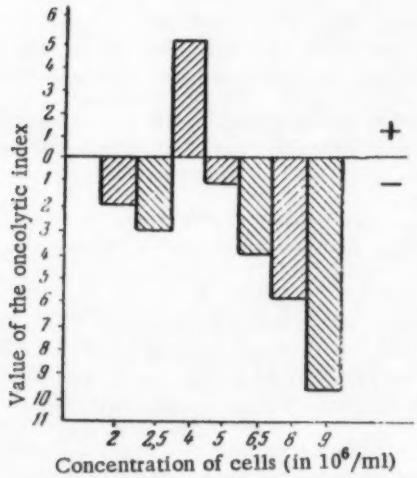

Fig. 3. Determination of the optical concentration of the suspension of tumor cells.

Measurement of the optical density of the medium contained in the hollows or tubes was carried out without delay after addition of the serum to the suspension of tumor cells, and then after different intervals of time through which the two were in contact with each other at 37° (equal volumes of serum and tumor-cell suspension were used). A portion of the tumor-cell suspension, added to Freund's solution, acted as a control. The tumor-cell suspension was prepared in the usual way [2]. The oncolytic index (OI) was calculated from the formula:

$$OI = \frac{OK_1}{O_1K},$$

where O and O_1 were the densitometer readings in the experiment before and after incubation respectively; K and K_1 were the control densitometer readings before and after incubation respectively.

The index was expressed by the number of hundredths by which the value exceeded unity or was less than unity. In the former, the index was preceded by a + sign, in the latter by a – sign. For example, 1.09 was expressed as +9; 0.88 as –12. This method of expression of the indices was adopted because in estimation of the oncolytic activity of the sera by the method of photometry, the absolute number of hundredths was of fundamental importance in our experiments.

In order to determine the optimal time of contact of the tumor cells with the serum, suspensions of tumor cells with serum (and also a control) were incubated for 30, 60, 90, 120, 150 and 180 minutes. The optical density was measured before and after incubation of the suspensions. From the results obtained, curves were drawn: along the abscissa was shown the time of contact, and along the ordinate, the densitometer readings with a coefficient of translucency equal to 100. For construction of the curves the results of experiments repeated not less than three times were selected. Theoretically the control curve should have shown the course of autolysis alone, and the experimental curve the course of autolysis of the cells plus that of specific oncolysis. Both the experimental and control curves should have reflected a continuous decrease in the optical density of the medium. Where the medium to be tested showed oncolytic activity, this decrease should have taken place more rapidly in the experimental test than in the control. The character of the curves was, however, much more complicated. Evidently the course of change in the optical density of the medium was influenced by agglutination, sedimentation and autolysis of the cells. All these factors complicated the curves and made their analysis more difficult. In order to simplify the analysis, model experiments were carried out,

for the purpose of obtaining curves showing changes in the optical density of the medium in cases where agglutination, sedimentation or autolysis of the cells was present in the "purest" form. These experiments greatly simplified the analysis of the complex curves which were obtained as a result of plotting the results of the series of experiments aimed at estimating the optimal times of incubation of the tumor-cell suspensions in the serum.

Altogether 11 experiments were performed, each of which was repeated from three to five times. In Fig. 2 are shown the curves of two typical experiments, reflecting the course of the change in the optical density of a suspension of tumor cells during incubation. Analysis of the curves showed that in the majority of cases the optimal time of incubation of the cells in serum was 60 minutes. This was also confirmed when the results obtained were tested for standardization. The limits of variation of the results, as a percentage of the mean OI, were least (26.1) in those cases when their standardization was tested after 60 minutes. In all the remaining cases the limits of variation were wider (from 28.1 to 44.6%).

Oncolytic Indices as Determined by the Cell Counting and Photometric Methods

Diagnosis of disease	OI, determined by the method of		Sera of healthy rabbits	OI, determined by the method of	
	cell counting	photometry		cell counting	photometry
Carcinoma of the stomach	0.50	−1	895	0.25	0
Carcinoma of the stomach	0.33	−6	151	1.10	+7
Chorionepithelioma	0.54	−13	152	3.30	+2
Healthy female	2.10	+8	152-A	2.80	+8
Healthy male	2.30	+9	897	1.92	+16

For determination of the optimal concentration of the suspension of tumar cells with the sera, suspensions of tumor cells of various concentrations were mixed. Readings were taken after incubation for 60 minutes. Altogether 10 experiments were performed. Each experiment was repeated not less than three times. The results of these experiments (Fig. 3) showed that the optimal concentration of the tumor cells was 4×10^6 per ml.

In order to find out if the results of the photometric method agreed in principle with those obtained by counting the cells, the oncolytic indices of five sera possessing oncolytic activity and of five sera known to be without such activity, were determined by both methods (see table).

As may be seen from the table, the results of both methods were in principle the same. The sera with no oncolytic activity in both cases gave oncolytic indices below unity, and those with oncolytic activity – above unity.

For a comparative estimation of the value of the two methods we compared the degree of standardization of the results obtained by each. For this purpose 15 experiments were carried out by the method of counting cells, 15 experiments in which the results were obtained by means of the "EEL" densitometer, and 15 experiments with our own densitometer. It was found that by the cell-counting method the limits of variation amounted to 30.39% of the mean value of the OI.

By the photometric method, by the use of the "EEL" densitometer the limits of variation amounted to 12%, and by the use of our own densitometer the limits were 16.94%. These results were treated statistically and were found to be significant ($p = 0.983$).

The information given shows that the method of determination of the oncolytic activity of sera, based on the principle of photometry, is simpler and less laborious than the method of counting cells. Furthermore, the results obtained by the use of the proposed method have a much higher degree of standardization. It may therefore be hoped that the method described may find application in oncology in the determination of the oncolytic properties of sera.

SUMMARY

The proposed method of the sera oncolytic activity determination by densitometers is based on the photometric principle. Optimal conditions for the oncolysis reaction have been established. In principle the photometric method renders results analogous to those obtained by counting the visibly unchanged cells, but is less cumbersome and its results are of a more standard nature.

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ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LÉM	Laboratory for Experimental Morphogenesis
LÉNDVI	Leningrad Inst. of Dermatology and Venereology
LÉO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIÉM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED

[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

* BRITISH-AMERICAN TRANSLITERATION SYSTEM.

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Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Eksptl. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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